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INVESTIGATIONS ON ESCHERICHIA COLI IN ACUTE APPENDICITIS AND IN NORMAL APPENDICES AND FAECES

WITH SPECIAL REFERENCE TO O GROUPS 1-25

BY

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PREFACE

The material examined in this study was collected at the Surgical Clinic of the University of Turku. Prof. A. R. Klossner, M.D., the Head of the Clinic and my former instructor, has shown great interest in my work and has continuously encouraged me. For all this I wish to express my sincere gratitude.

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Turku, January, 1958.

Matti K. Leppänen

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INTRODUCTION

The opinions on the origin of acute appendicitis presented in modern textbooks differ considerably. Primarily on the basis of experimental investigations, some of which have been performed on humans, the conclusion has been drawn that appendicitis results from the combined effect of lumen obstruction, bacterial infection, and increased intraluminal pressure (Wangensteen et alii 1934, 1937, 1939, 1940, Eichhoff and Pfannenstiel 1931, Farris and Romack 1947, Nigam 1947, Huber 1950, Schönbauer 1951).

The bacterial flora associated with acute appendicitis and appendical peritonitis has been extensively studied since the last century. Numerous micro-organisms have been isolated, but a common finding has been that the following three genera have been most frequently encountered: Escherichia, Streptococcus and Clostridium.

The micro-organism isolated in the majority of cases has been Escherichia coli. In different series this micro-organism has been encountered in 65 to 100 per cent of cases. The opinions of the part played by Escherichia have varied greatly. Several investigators, especially those who worked at the turn of the century and a few who have conducted experimental studies in recent years, have considered this bacterium, either alone or together with other micro-organisms, the primary cause of acute appendicitis. On the other hand, many other authors have considered it a co-existing organism without pathogenic properties.

Escherichia coli and the other bacteria that have been isolated from inflamed appendices are normally present in the intestine. The question whether the Escherichia strains encountered in inflamed and healthy appendices differ in character has not yet been decisively answered. After Kauffmann and his co-

workers had discovered the antigenic structure of Escherichia coli and developed the antigenic schema for this group, it became possible to define more accurately the Escherichia florae present in healthy and diseased appendices. The number of published papers dealing with this problem are yet few in number and for this reason it was thought appropriate to undertake a study of the occurrence of certain Escherichia coli serotypes in acute appendicitis.

PREVIOUS STUDIES

INVESTIGATIONS RELATING TO THE SEROLOGY OF ESCHERICHIA COLI

Smith had observed even as early as 1927 that a thermostable antigen prevented the agglutination of living capsular Escherichia coli strains in O immune sera prepared with acapsular forms, but the foundation for the antigenic schema of Esherichia coli was laid when Kauffmann discovered the thermolabile L antigen in 1943. Previous work on the serology of E. coli (Durham 1897, Burk 1908, Bitter and Gundel 1925, Hees 1926, Mikkelsen 1927, Hayashi 1938) had been unsuccessful owing to the fact that the L antigen prevented the agglutination of living and formalinized Escherichia strains. Only after the culture is heated, preferably at 100°, for 1—2 hours, does the O inagglutinability disappear and the strain is agglutinated by the homologous O serum (prepared using the boiled strain) with a titre of 1:5000 or higher.

According to Kauffmann the antigenic structure of Escherichia coli comprises O, K and H antigens of which either or both of the latter two may be absent. The O antigen is a thermostable somatic antigen. The K antigen is a common designation for the envelope and capsular antigens (Kauffman and Vahlne 1945). The A antigens discovered by Kauffmann in 1944 are thermostable capsular antigens (Knipschildt 1945). Strains which have an A antigen lose their O inagglutinability only after they have been autoclaved at 120° for 2 hours (Vahlne 1945). The L and B antigens are thermolabile envelope antigens which are destroyed by heating 1—2 hours at 100°. The existence of the B antigens was demonstrated by Knipschildt (1945). These differ from the L antigens by retaining their antibody-binding properties in spite of heating to 100° and from the A antigens by losing their O-agglutination-inhibiting property on heating

to 100°. The H antigens are thermolabile flagellar antigens and according to Kauffmann (1954) monophasic.

Kauffmann (1944) demonstrated the existence of 58 different O antigens and 17 different L antigens. On this basis, he developed his antigenic schema comprising 20 different O groups which were divided into subgroups according to their L antigens. Knipschildt (1945) discovered 52 new O antigens, 7 L antigens, 12 A antigens and 3 B antigens. Following the investigations of Vahlne (1945), the antigenic schema comprised 25 O groups which were subdivided further according to the K and H antigens.

At present 137 different O antigens, 79 K antigens, and 40 H antigens are recognized (Ewing et al. 1956).

Studies of the pathogenicity of Escherichia coli by Kauffmann (1954), Knipschildt (1945), Vahlne (1945), Ewertsen (1946) and Sjöstedt (1946) have led to the following conclusions:

1. Escherichia strains from pathological processes generally belong to a limited number of O groups, 62—78 per cent to the first 25 O groups, to which only about half the strains of faecal origin belong.

2. Pathological material is serologically much more uniform than faecal material. All strains found in the former belong in most cases to one serological O group; in the latter, strains belonging to as many as 12 different O groups have been isolated from the same specimen.

3. Strains from pathological processes generally are capsular, while the capsule is frequently absent from the faecal strains.

4. The toxicity of E. coli is associated with the K antigen.

5. The toxicity is largely dependent on the ability of the strain to effect haemolysis and necrosis.

6. The necrotizing action varies in different O groups.

7. Necrotizing strains are often haemolytic.

8. Haemolytic power is a property possessed by strains of only a few groups, particularly O groups 2, 4 and 6.

9. The capsular strains are more resistant to the bactericidal action of human serum than acapsular strains.

10. The capsular strains are not as sensitive to phagocytosis as the acapsular strains. E. coli O groups especially pathogenic to man and animals are the groups 1, 2, 4, 6, 8 and 9.

Kauffmann (1944) ascribed the following biochemical properties to E. coli strains: they form indole, do not attack urea, do not liquefy gelatin and give a negative Voges-Proskauer test and a positive methyl red reaction. He stated that the strains may exceptionally be lactose-negative and may grow in ammonium substrates containing sodium citrate. If these strains exhibited the other properties attributed to E. coli strains, they were considered to belong to this group. By examining their ability to utilize adonitol, dulcitol, inositol, rhamnose, salicin, sucrose and xylose, it was possible to assign the strains to numerous biochemical types. Kauffmann and Perch (1948) established that the biochemical behaviour of different Escherichia coli strains is constant.

THE OCCURRENCE OF E. COLI SEROGROUPS IN HEALTHY AND INFLAMED APPENDICES AND IN FAECES

E. coli is rarely found in the acid gastric juice (Henning 1930, Korttila 1951, Levanto 1954). Levanto determined the antigenic structures of 82 E. coli strains he isolated from achlorhydric gastric juices. Twenty-seven (33 %) of the strains belonged to the O groups 1—25, but no serotype predominated over the others.

In contrast, E. coli is a permanent inhabitant in the large intestine. In previous studies of the bacteriology of appendicitis it has been observed that Escherichia coli is almost always the bacteria that is isolated in the highest frequency. Various authors have found this bacterium in 65—95 per cent of their cases of appendicitis (Kelly 1899, Krogius 1900, 1901, Lanz and Tavel 1904, Runeberg 1908, McWilliams 1910, Vick 1912, Dudgeon and Mitchiner 1923, Bagger and Mikkelsen 1924, Warren 1925, Weinberg, Pierot and Davesne 1928, Aschoff 1930, Hirose 1930, Löhr and Rassfeld 1931, Meleney et al. 1931, 1932, Kemal 1934, McClure and Altemeier 1937, Balice 1937, Altemeier 1938, Bower, Burns and Mengle 1938, Husted 1942, Diaz Martin 1946). Ewertsen and Knipschildt (1946) found Escherichia coli strains in the lumens of every one of the 187 inflamed appendices they examined. Löhr and Rassfeld (1931) and Eichhof and Pfannenstiel (1931) had found previously that

the frequency of E. coli did not differ in inflamed and normal appendices.

Vahlne examined in 1945 a very extensive material comprising 583 appendices. He found no E. coli strains in 48 of these. Of the remaining appendices he considered on the basis of macroscopic observation 429 to be clearly appendicitic and 106 suspectedly appendicitic. In addition he isolated E. coli strains from 128 exudates from peritonitis patients. The inflamed appendices of thirty-three of these patients were examined for the presence of Escherichia strains. In addition, he examined 156 normal appendices; of these 23 did not contain E. coli strains. Faecal samples obtained from fifty appendicitis patients contained E. coli, while E. coli was cultured from all but 23 faecal samples from 501 faecal controls.

Some of the results of Vahlne have been collected in Table 1 which shows (a) the O groupability of the strains, (b) the specimens from which O-groupable strains were isolated, and

TABLE 1

OCCURRENCE OF ESCHERICHIA COLI ACCORDING TO VAHLNE (1945).

Material	Specimens containing E. coli strains	No. of strains examined	O-groupable strains in per cent	Percentage of cases with O-groupable strains	Percentage of cases with all strains of the same O group
Normal faeces	392	1 414	42	61	27
„ appendix	133	513	68	81	46
Appendicitis	429	1 663	78	87	53
Appendical peritonitis	128	507	73	81	63
Faeces from non-appendicitic patients	86	349	52	76	28
Faeces from appendicitis patients	50	195	55	78	21
Suspected appendicitis	104	416	75	83	56
Urinary infections	142	406	61	60	95
Cholecystitis	49	179	44	49	74
Total	1 513	5 542	64	74	50

(c) the specimens from which all strains isolated belonged to the same O group. From his data, Vahlne concluded that "serologically the colon flora is more polymorphous in normal material than in pathological material. This is evident from the fact that the colon strains isolated from the various cases more often belong to the same group in the pathological material than in the normal material; and furthermore, a considerably higher percentage of the former can be entered in the O groups of the antigenic schema".

Vahlne observed the following variations in O inagglutinability: 65 per cent of the faecal strains, 75 per cent of the strains from uninflamed appendices, 90 per cent of appendicitis strains, and 87 per cent of the peritonitis strains were O-inagglutinable. His strains did not enter into all 25 O groups, but only into eight most frequently occurring O groups, viz., 1, 2, 4, 6, 8, 9, 18 and 21. None of the O groups predominated over the other groups in the pathological or normal material. In 13 of 50 cases strains of the same type or of the same O group were found in both the faeces and inflamed appendices. In 21 of 33 cases, strains of the same O group were isolated from the appendices and peritoneal exudates; in most cases the strains possessed also the same K and H antigens. Nineteen per cent of the peritonitis strains, 10 per cent of the appendicitis strains, and 9 per cent of the strains from faeces specimens and uninflamed appendices were haemolytic.

Vahlne's opinion was that the bacterial florae in E. coli infections are of intestinal origin. However, only a few of the Escherichia strains are sufficiently resistant to the defensive forces of the organism to be capable of further growth in the new milieu. This resistance can be primarily attributed to the presence of a capsule and to the ability to haemolyse.

In a later paper (1945) Vahlne concluded that practically all O groups are represented in both pathological and normal appendices. No greater differences are noted between the two types of material if only the O antigen is taken into account. When he examined the occurrence of different serotypes of the most common O 9 group in pathological and normal material, he isolated the serotype 9:34:— from 16 pathological and from 3 healthy appendices and the serotype 9:36:19 from 10 pathological appendices

but from none of the healthy appendices. On the other hand, acapsular strains of group O 9 he found in 18 healthy appendices but in none of the diseased appendices. On the basis of this examination of the occurrence of strains of the group O 9, he concluded that the pathological strains are of certain definite types while most of the strains isolated from normal material do not possess a K antigen. In conclusion, he stated that the E. coli strains in pathological and healthy appendices differ in their serological properties and that certain E. coli serotypes are most frequently present in appendicitis.

Ewertsen and Knipschildt (1946) found E. coli in all 187 cases of appendicitis they examined, in 4 of these as a pure culture. 316 of 556 peritoneal exudate samples were found sterile. E. coli was isolated as a pure culture from 99 of the remaining specimens and together with other bacteria from 121 specimens, but was absent from 20 specimens. Seventy-four per cent of the appendicitis strains belonged to the 20 most common O groups (O groups 1—12, 15, 18, 21—25 and 29). Seventy-five per cent of the peritonitis strains, 65 per cent of the strains from uninflamed appendices (113 cases), and 51 per cent of the faecal strains (197 cases) belonged to these groups. Ewertsen and Knipschildt came to the conclusion that although strains of the 20 most common O groups were somewhat more frequently isolated from appendicitis and peritonitis than from faecal specimens, it is not justified to consider them particularly pathogenic.

Mondolfo and Hounie (1947) examined 1065 Escherichia strains from various sources; of these 692 entered the first 25 O groups. The O group was determined for 388 of 600 faecal strains from normal subjects, and for 156 of 200 faecal strains from patients with intestinal disease. The most common O groups were 2, 4, 5, 7, 8, 9, 15, 18, 25. Their final conclusion was that the same E. coli O groups prevail in Argentina as in Europe.

In a study of 225 E. coli strains from 60 inflamed appendices and 10 faecal specimens, Scire (1950) established that 46.7 per cent of the appendicitic and 61.2 per cent of the faecal strains entered O groups 1—25.

Soave (1950) examined 50 cases of appendicitis of varying severity and found that capsular strains were more frequently present in severe inflammations.

Parvis and Grosso (1953) examined 333 Escherichia strains isolated from appendices of persons with appendicitis and peritonitis and from appendices of healthy persons. They found that 65 per cent of the appendicitis strains, 53 per cent of the peritonitis strains, 36 per cent of the strains from uninflamed appendices, and 24 per cent of the strains from faecal specimens from healthy persons belonged to the O groups 1—25. They also found several strains that could not be classified according to the antigenic schema.

Kubinyiné-Schwanner and Hamar (1955) isolated 300 E. coli strains. Forty-four of 100 strains isolated from inflamed appendices, 33 of 100 faecal strains and 52 of 100 urinary strains could be entered into the first 25 O groups.

Kauffmann (1948) has concluded that appendicitis is an endogenous infection. Certain serological types possessing the K antigen are present in normal appendices. According to Kauffmann, these strains must be considered important predisposing agents. The agent causing the inflammation is thus always present and may become pathogenic as soon as the appendical mucosa suffers injury. If the Escherichia strains possess necrotizing properties, gangrene usually develops.

According to Kauffmann, haemolytic streptococci and pneumococci cannot be aetiological factors in appendicitis since they have been isolated from only a few of the hundreds of appendices examined. The part played by enterococci is still uncertain; their importance as aetiological factors can be assessed only after they have been subdivided into serological groups. Kauffmann has further remarked that although E. coli must be considered the main causative agent of appendicitis and of peritonitis of appendical origin, the possible significance of enterococci and other, mainly anaerobic, bacteria must not be ignored.

Ørskov (1956) studied 581 E. coli strains isolated from 581 infants with diarrhoea and 183 strains isolated from 183 healthy infants using 130 O immune sera. In the former group he found 453 strains that belonged to 69 different O groups and in the latter group 151 strains that belonged to 45 different O groups. Thirty-eight per cent of the diarrhoeal strains and 40 per cent of the strains from the healthy infants could be entered into the O groups 1—25. No O group was observed more frequently than

the others. Ørskov noted also that strains belonging to the same O group very often differed in their biochemical behaviour.

Grönroos (1957) has also investigated the incidence of strains of the O groups 1—25 in the faeces of healthy infants (2147 strains) and of 63 diarrhoeal infants (498 strains). Strains groupable with O immune sera 1—25 amounted to 32.9 ± 1.0 per cent of the strains of the former group and to 29.2 ± 2.3 per cent of the strains of the latter group. Only 47.8 ± 1.8 per cent of the strains of O groups 1—25 could be typed by means of the antigenic schema of Kauffmann—Knipschildt—Vahlne. The most common O groups were 1, 2, 4, 6, 8, 17 and 18. Strains without K antigens amounted to 8.5 ± 1.0 per cent of the strains isolated from healthy infants, while no acapsular strains were isolated from the diarrhoeal infants. In only 8.6 ± 2.0 per cent of the faecal specimens were all the isolated Escherichia strains of the same serotypes. When only the O antigen was noted, all strains isolated belonged to the same O group in 17.6 ± 2.8 per cent of the specimens. No significant differences were observed in these respects between the healthy and diarrhoeal infants.

Wramby (1948) found that 40 per cent of 5961 E. coli strains he had isolated from the intestines of healthy calves and from the intestines, lymph nodes, cerebral ventricles, livers, heart blood and spleens of calves suffering from E. coli septicaemia (white scours) belonged to the O groups 1—25. When he compared his results with those of Vahlne, he found that there are great dissimilarities as regards the frequency of the E. coli strains of different O groups; only the frequencies of the O 9 group strains were equal in the two series. The frequencies differed most for the O groups 8 and 15, strains of which were more often isolated from calves than from humans. Only 21.5 per cent of Wramby's strains possessed a K antigen.

Fey (1955) found among 1806 strains isolated from faeces of cows only 90 strains that belonged to O groups 2, 6, 8, 9 and 21.

PREVIOUS STUDIES ON THE OCCURRENCE OF E. COLI AGGLUTININS IN THE SERA OF APPENDICITIS PATIENTS

As the detection of agglutinins in the serum of a patient is of decisive significance for the evaluation of the part played by a bacterium in causing disease, many attempts have been made to detect antibodies for Escherichia strains in the sera of patients suffering from appendicitis and appendical peritonitis (Runeberg 1908, Heile 1913, Dudgeon and Mitchiner 1923, Neter and Milch 1940) but even the highest recorded titres have been low (1:200). It is understandable that the demonstration of antibodies was unreliable since the antigenic structure of Escherichia was not known to these investigators.

Vahlne was the first to study antibody production in cases of appendicitis after the elaboration of the Escherichia antigenic structure. The study involved 94 patients with established appendicitis from whom serum samples were taken on the sixth postoperative day. The O and H antibodies in the sera were investigated using the homologous E. coli strains isolated from the appendices of the patients. Increased antibody titres were obtained for only 11 of the 94 sera. The highest O antibody titre was 1:640 (4 cases) and the highest H antibody titre 1:12,800 (1 case). Vahlne stated that "these findings indicate that uncomplicated appendicitis may be associated with an antibody production against the colon bacteria occurring in the appendix".

Vahlne looked for antibodies in 203 control sera using fifteen O and fifteen H antigens. In 51 cases the titre was high, but not higher than 1:160 for an O antigen, nor higher than 1:1,280 for an H antigen.

Kröger and Gillesen (1950) did not observe increased E. coli antibody titres in their appendicitis patients.

Neter, Bertram and Arbesman (1952) and Neter, Bertram, Zak, Murdock and Arbesman (1952) were able to demonstrate E. coli O 55 and O 111 antigens by haemagglutination tests. They found that red blood cells of man, rabbit, guinea-pig, sheep, rat and chicken absorb E. coli O 55 and O 111 antigens (boiled strains) and thus become specifically agglutinable by homologous E. coli antisera.

Neter, Gorzynski, Zalewski, Rachman and Gino (1954)

established in 18 patients with appendical peritonitis, from each of whom at least two blood samples had been taken, an increase in homologous E. coli haemagglutinins ranging from four-fold to thirty-fold in ten, a two-fold increase (titre 1:160) in five, and no increase in three patients. Since they were able to demonstrate bacterial afflutinins (with low titres 1:20, 1:40) in only three patients, they concluded that haemagglutination is a more sensitive indicator of antibodies than the bacterial agglutination test.

When examining the increase in haemagglutinins in the sera of eight patients with uncomplicated appendicitis, these same authors established a four-fold or greater increase for the homologous strain isolated from the appendix in three cases, a two-fold increase in two, and no increase in three cases. In the cases where no increase was observed, the haemagglutinin titre was in their opinion relatively high, 1:160 or higher. Bacterial agglutinin titres 1:20 and 1:80 were found for the sera of only two of the eight patients.

Kubinyiné-Schwanner and Hamar (1955) succeeded in demonstrating haemagglutinins in the sera of only two of eight appendicitis patients.

SUMMARY

In the elaboration of the antigenic structure of the Escherichia coli group in the 1940's, Kauffmann and his coworkers found the following properties of E. coli strains to be correlated: O group, O inagglutinability, origin, toxicity and haemolytic and necrotizing powers. Accordingly, strains isolated from pathological processes (appendicitis, peritonitis, urinary infections, cholecystitis) belong to a small number of O groups and are more often O-inagglutinable, haemolytic, necrotizing and toxic than strains of faecal origin. When it was further established that strains isolated from the same inflamed appendices or peritonitis specimens were more uniform, i.e. belonged to the same O group, than faecal strains, Kauffmann concluded that the capsular Escherichia coli strains are the primary causative agents of appendicitis. On the other hand, it has not been possible to show that strains of one O group, even less some definite serotypes.

are more often associated with the diseases mentioned. It has been observed, however, that the E. coli strains isolated from pathological material can be more often entered into the O groups 1—25 of the antigenic schema of Kauffmann-Knipschildt-Vahlne than strains of faecal origin.

The existence of bacterial agglutinins against Escherichia coli in the sera of patients with uncomplicated appendicitis could be demonstrated only after the elucidation of the antigenic structure. The antibody titres have generally been low. Agglutinins have been detected for O and H antigens but not for K antigens. When the presence of haemagglutinins for Escherichia coli has been examined in peritonitis patients, the titres have been observed to increase with time.

THE PROBLEMS

1. Do the Escherichia coli florae of diseased and healthy appendices and faecal specimens of appendicitis patients and healthy persons differ?

2. Do the Escherichia coli strains in the lumen and wall tissue of an appendix, in peritoneal exudate, in the region proximal to lumen obstruction in an appendix, and in the regional lymph nodes differ in their serological characteristics?

3. Do the Escherichia florae of histopathologically different forms of appendicitis differ?

4. Are agglutinins for homologous Escherichia coli strains found in the sera of appendicitis patients?

THE PRESENT INVESTIGATIONS

METHODS

COLLECTION AND TREATMENT OF SPECIMENS

The appendices examined were from patients operated upon at the Surgical Clinic of the University of Turku. Whenever a patient on whom appendicectomy was being performed had exudate in the abdominal cavity, a specimen was taken into a sterile test tube immediately on entry. The excised appendix and, when present, an ileocoecal lymph node were transferred to separate sterile Petri dishes. The specimens were in most cases taken immediately to the Department of Medical Microbiology located nearby and cultured. In a few cases the bacteriological examination of the specimens was carried out on the following day; in the meantime, the Petri dishes containing the specimens were stored in a refrigerator at +5°. Faecal specimens were taken in connection with the first voiding after the operation, in most cases on the third day, by which time some of the patients, especially the peritonitis cases, had been given antibiotics, usually penicillin and streptomycin intramuscularly, and in a few cases sulphonamides intravenously. Blood samples required for the agglutinin determinations were withdrawn on the day of discharge, usually on the 5th to 7th day after the operation. The centrifuged sera were stored in sterile test tubes in a refrigerator at —20°C until they were examined.

The serosa of the excised appendix was cleaned with 94 per cent alcohol in order to destroy bacteria transferred with peritoneal exudate. A swab specimen was then taken from the serosa for culture. If bacterial growth resulted, the next specimen taken from the appendix wall was discarded. This tissue specimen comprising an area 5 mm square of serosa, muscularis and submucosa was detached with sterile instruments after the alcohol treatment; care was taken not to puncture the mucosa. If the mucosa did not remain intact, the specimen was discarded. The tissue specimen was placed in liver broth. Samples were taken with a platinum loop from the exposed area of the appendix wall and transferred to plates.

When a faecalith was found that divided the appendix into a distal, inflamed region and a proximal, macroscopically "uninflamed", region, the latter was immediately cut off to enable a sample to be taken from its lumen.

The appendix was slit lengthwise with sterile instruments, and a lumen sample was taken from the most inflamed region of the mucosa. The lymph nodes were dipped in 94 per cent alcohol and their surfaces sterilized in a flame, after which they were cut into small pieces and placed in liver broth.

CULTURE METHODS AND IDENTIFICATION OF STRAINS

The following media were employed in the culture of the samples:

1. Bromcresol purple lactose agar:
   Meat extract 1000 ml, peptone 5 g, agar 20 g, lactose 15 g, bromcresol purple (1.6 per cent solution in alcohol) 1 ml.
2. Blood agar: 5 per cent sheep's blood in agar medium of pH 7.2—7.3.
3. Liver broth: 5—6 pieces (3—4 g) of fresh liver in broth, pH 7.2—7.3.

The tissue specimens removed from the appendix walls and the minced lymph nodes were cultured in liver broth. The samples taken with platinum loops from other sites were inoculated onto plates and into liver broth and incubated overnight at 37°. If no growth was observed after 24 hours, the plates were kept two days and the liver broth cultures three days at 37°. Subcultures were then transferred from the liver broth to plates.

Five colonies from each of the subcultures of specimens from the appendix wall, from the lumens of the distal and proximal regions of the appendix, from the peritoneal exudate, from the lymph node and from the faeces were examined.

The following biochemical reactions of the strains were investigated: mannitol (Durham tube) and lactose fermentation, indole production, hydrogen sulphide production, liquefaction of gelatin, urea decomposition, citrate utilization, the methyl red reaction and the Voges-Proskauer reaction. The media employed in these biochemical tests and the procedures followed are those currently used at the Department of Medical Microbiology and have been previously described by Grönroos (1954).

The above biochemical tests were chosen on the basis of Kauffmann's definition of Escherichia coli: "a large species of serologically related, gram-negative, non-sporing rods showing, with certain exceptions, a motile peritrichous phase in which they normally occur. They usually form indole and give a negative Voges-Proskauer test, but a positive methyl red reaction. They do not decompose urea, and usually they do not utilize ammonium citrate. Gelatin is not liquefied."

Only in cases where the results of the above tests were not typical of Escherichia coli was the utilization of the following sugars examined: adonitol, dulcitol, inositol, salicin and sucrose.

The motilities of the strains were studied in Craigie tubes (Craigie 1931) at 37°. If the strain did not pass through the medium in 24 hours, the tube was kept at room temperature for two weeks. It was then inoculated into meat broth, incubated 24 hours at 37°, and its motility again examined after transfer to a Craigie tube. If no passage occurred, the tubes were stored at room temperature for a further two weeks, the culture was inoculated into meat broth and again transferred to a Craigie tube. If no passage even now occurred, the strains was judged non-motile.

The haemolytic powers of the E. coli strains were tested according to Widholm (1953). Two per cent defibrinated sterile sheep red cells were added to a tube containing 1 per cent peptone water of pH 7.6. The volume of the medium in each tube was usually 2 ml. After the medium had been inoculated with a loopful of bacterial culture and incubated overnight at 37°, the tubes were examined for haemolysis.

SEROLOGICAL TECHNIQUE

The type strains were all obtained from the International Salmonella and Escherichia Coli Centre through the courtesy of Drs. F. Kauffmann and F. Ørskov.

Preparation of antigens. O antigen. For the preparation of O immune sera, overnight broth cultures were steamed for 2½ hours. The antigens so prepared were also used for the identification of the O antigens of the strains isolated from the specimens. In addition, a second tube of the overnight broth culture was autoclaved two hours at 120° to destroy any A antigen possibly present. The O antigens prepared in this way were examined with only the mixed serum E (see below).

K antigen. Living strains possessing an L, A or B antigen were employed in the agglutination studies and in the preparation of immune sera. A subculture was inoculated onto a 0.1 per cent glucose slant agar from an 18-hour-old 0.1 per cent glucose agar, and after overnight growth the resulting culture was suspended in 5 ml of physiological saline. The K antigen suspensions were prepared immediately before use.

H antigen. Formolized overnight broth cultures of very motile strains were employed for immunization and antigen identification.

Preparation of immune sera. Rabbits approximately one year old were used in the preparation of immune sera. The antigen was injected in increasing amounts, 0.25, 0.5 and 1.0 ml, into the marginal ear vein of a rabbit at intervals of four days. The first sample was taken on the fourth day following the last injection. If the titre was sufficiently high, the rabbit was bled by dividing the carotic artery; if not, the immunizations were continued with 1.5- and 2.0-ml antigen injections. The serum was isolated after the coagulum had retracted and the blood had been centrifuged. After merthiolate (Lilly) had been added, the

serum was transferred to sterile flasks and inactivated thirty minutes at 56°. The flasks were then stored at 4°.

For the antigen studies, immune sera for the antigens O 1—25, K 1—55 and H 1—33 were prepared using type strains.

Determination of the antigenic structures of the E. coli strains. For the O antigen determinations, the type sera were combined into five groups, each containing five different sera. Each group comprised those sera which gave the highest overlapping reactions with each other (for a table of overlapping reactions, see Grönroos 1957); in this way interfering overlapping reactions between the groups were largely avoided.

The mixed sera employed were the following:

A	containing	O	sera	1, 2, 3, 10, 23;
B	„	„	„	4, 13, 18, 19, 25;
C	„	„	„	5, 6, 7, 12, 15;
D	„	„	„	11, 16, 20, 21, 24;
E	„	„	„	8, 9, 14, 17, 22.

The proportions of the component sera were chosen on the basis of their titres. The mixed sera were diluted 1:100.

The determination of the O antigen was performed in three stages. In the first stage, mixed sera were employed. In the second stage, the individual components of the mixed serum that yielded a positive agglutination test were used. In both these stages dilution series consisting of three 0.2-ml tubes were employed for the determination of the antigen group and antigen as described by Grönroos (1957). In the third stage the final titres were determined using 0.2-ml volumes. The strain under study was considered to belong to a certain O group if the titre obtained with it was the same as the titre of the immune serum for the homologous type strain, usually 1:5000 or higher. Deviations of one tube were permitted.

As mentioned in connection with the preparation of the O antigens, each strain examined was autoclaved two hours at 120° to destroy the A antigen possibly present and studied using mixed serum E, since the latter contained sera for O groups 8 and 9 which according to the antigenic schema contain only strains that possess the A antigen.

The K antigens were determined in the following manner. The O inagglutinability of the strain in question was first determined on a slide using the living strain and undiluted O serum. When the study was begun, the O-inagglutinability tests were performed after the slide tests with both the living and boiled strain using immune serum containing the O antigen which the strain in question had been found to possess. If the titre was high in both tests (living and boiled strains), the strain was not considered O-inagglutinable. When the titre for the living strain was lower than 1:160, the strain was taken to be O-inagglutinable (Kauffmann 1944). Since it only seldom happened that a strain which agglutinated on a slide gave a titre below 1:160 in the tube test, the slide agglutination test was subsequently considered

to yield an adequate indication of O inagglutinability. Vahlne (1945) came to the same conclusion in his studies. If the strain was completely O-inagglutinable, tests were made to determine whether the strain possessed one of the K antigens associated with its O antigen according to the antigenic schema using OK sera in 1:2 dilution and living strains. Also these agglutination tests were performed on slides. If no K antigen suggested by the antigenic schema was detected, identification of the K antigen was attempted using all the other OK sera (1—55). All positive results obtained in the slide agglutination tests were checked by tube tests.

All strains for which the O antigen could not be determined were nevertheless examined for their K antigens using all OK sera available, first on slides, and if positive, in tubes. Whenever a K antigen was identified in these tests, the strain was again examined using all O sera which are associated with the identified K antigen in the antigenic schema.

The following mixed sera were used in the identification of the H antigens:

A	containing	H	sera	1, 2, 4, 6, 12;
B	„	„	„	5, 7, 9, 10, 13;
C	„	„	„	11, 14, 15, 16, 21;
D	„	„	„	17, 18, 19, 20, 27;
E	„	„	„	3, 8, 22, 23, 24;
F	„	„	„	25, 26, 29, 31;
G	„	„	„	28, 30, 32, 33.

The sera were grouped primarily according to the level of their titres, since Kauffmann has stated that overlapping reactions of H sera are seldom observed. The titres were determined in the first and second stages using 0.2-ml test tubes and the final titres using 0.2-ml volumes.

The results of the O agglutination tests were recorded after the test tubes had been in a water bath at 50° for 20 hours.

The L and B tube agglutinations were read after the tubes had been 2 hours at 37° and then overnight at room temperature. The A antigen tests were performed after the tubes had been kept in a water bath at 50° for 2 hours.

The H tube agglutinations were read after two-hour incubation in a water bath at 50° (Kauffmann 1954).

Storage of strains. The strains were stored in egg culture medium (Kauffmann 1941) in the dark at room temperature.

DETECTION OF AGGLUTININS IN THE SERA OF PATIENTS

Bacterial agglutination tests. For the identification of agglutinins possibly present in the sera of the patients, ordinary bacterial agglutination tests were performed with O, K and H antigens starting with a dilution of 1:20. The bacterial agglutinations were performed in two different

The appendix was slit lengthwise with sterile instruments, and a lumen sample was taken from the most inflamed region of the mucosa. The lymph nodes were dipped in 94 per cent alcohol and their surfaces sterilized in a flame, after which they were cut into small pieces and placed in liver broth.

CULTURE METHODS AND IDENTIFICATION OF STRAINS

The following media were employed in the culture of the samples:

1. Bromcresol purple lactose agar:
   Meat extract 1000 ml, peptone 5 g, agar 20 g, lactose 15 g, bromcresol purple (1.6 per cent solution in alcohol) 1 ml.
2. Blood agar: 5 per cent sheep's blood in agar medium of pH 7.2—7.3.
3. Liver broth: 5—6 pieces (3—4 g) of fresh liver in broth, pH 7.2—7.3.

The tissue specimens removed from the appendix walls and the minced lymph nodes were cultured in liver broth. The samples taken with platinum loops from other sites were inoculated onto plates and into liver broth and incubated overnight at 37°. If no growth was observed after 24 hours, the plates were kept two days and the liver broth cultures three days at 37°. Subcultures were then transferred from the liver broth to plates.

Five colonies from each of the subcultures of specimens from the appendix wall, from the lumens of the distal and proximal regions of the appendix, from the peritoneal exudate, from the lymph node and from the faeces were examined.

The following biochemical reactions of the strains were investigated: mannitol (Durham tube) and lactose fermentation, indole production, hydrogen sulphide production, liquefaction of gelatin, urea decomposition, citrate utilization, the methyl red reaction and the Voges-Proskauer reaction. The media employed in these biochemical tests and the procedures followed are those currently used at the Department of Medical Microbiology and have been previously described by Grönroos (1954).

The above biochemical tests were chosen on the basis of Kauffmann's definition of Escherichia coli: "a large species of serologically related, gram-negative, non-sporing rods showing, with certain exceptions, a motile peritrichous phase in which they normally occur. They usually form indole and give a negative Voges-Proskauer test, but a positive methyl red reaction. They do not decompose urea, and usually they do not utilize ammonium citrate. Gelatin is not liquefied."

Only in cases where the results of the above tests were not typical of Escherichia coli was the utilization of the following sugars examined: adonitol, dulcitol, inositol, salicin and sucrose.

The motilities of the strains were studied in Craigie tubes (Craigie 1931) at 37°. If the strain did not pass through the medium in 24 hours, the tube was kept at room temperature for two weeks. It was then inoculated into meat broth, incubated 24 hours at 37°, and its motility again examined after transfer to a Craigie tube. If no passage occurred, the tubes were stored at room temperature for a further two weeks, the culture was inoculated into meat broth and again transferred to a Craigie tube. If no passage even now occurred, the strains was judged non-motile.

The haemolytic powers of the E. coli strains were tested according to Widholm (1953). Two per cent defibrinated sterile sheep red cells were added to a tube containing 1 per cent peptone water of pH 7.6. The volume of the medium in each tube was usually 2 ml. After the medium had been inoculated with a loopful of bacterial culture and incubated overnight at 37°, the tubes were examined for haemolysis.

SEROLOGICAL TECHNIQUE

The type strains were all obtained from the International Salmonella and Escherichia Coli Centre through the courtesy of Drs. F. Kauffmann and F. Ørskov.

Preparation of antigens. O antigen. For the preparation of O immune sera, overnight broth cultures were steamed for 2½ hours. The antigens so prepared were also used for the identification of the O antigens of the strains isolated from the specimens. In addition, a second tube of the overnight broth culture was autoclaved two hours at 120° to destroy any A antigen possibly present. The O antigens prepared in this way were examined with only the mixed serum E (see below).

K antigen. Living strains possessing an L, A or B antigen were employed in the agglutination studies and in the preparation of immune sera. A subculture was inoculated onto a 0.1 per cent glucose slant agar from an 18-hour-old 0.1 per cent glucose agar, and after overnight growth the resulting culture was suspended in 5 ml of physiological saline. The K antigen suspensions were prepared immediately before use.

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The determination of the O antigen was performed in three stages. In the first stage, mixed sera were employed. In the second stage, the individual components of the mixed serum that yielded a positive agglutination test were used. In both these stages dilution series consisting of three 0.2-ml tubes were employed for the determination of the antigen group and antigen as described by Grönroos (1957). In the third stage the final titres were determined using 0.2-ml volumes. The strain under study was considered to belong to a certain O group if the titre obtained with it was the same as the titre of the immune serum for the homologous type strain, usually 1:5000 or higher. Deviations of one tube were permitted.

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DETECTION OF AGGLUTININS IN THE SERA OF PATIENTS

Bacterial agglutination tests. For the identification of agglutinins possibly present in the sera of the patients, ordinary bacterial agglutination tests were performed with O, K and H antigens starting with a dilution of 1:20. The bacterial agglutinations were performed in two different

ways using two identical dilution series for each antigen. One of the series was tested as described above in the case of the O, K and H agglutination tests. The other dilution series for the determination of the O antigen was kept two hours in a 50-degree water bath, and that for the determination of K antigen two hours in a 37-degree water bath, after which they were centrifuged 10 minutes at 3000 r.p.m. (Braun et al. 1954). Already Gaethgens (1906), De Gara (1939), and Graber and Bonnefoi (1946) had found the centrifuge method to be more sensitive than the ordinary bacterial agglutination.

Both of the above methods were employed concurrently when examining the sera of patients for the presence of agglutinins. The presence of antibodies homologous with the appendical strains was determined in the sera of 92 patients and the presence of antibodies homologous with the immunization strain in the sera of 99 rabbits. The presence of both O and K antibodies was examined. The results of the ordinary bacterial agglutination tests and those of the tests made by the centrifuge method are shown in Table 2. The total number of parallel titrations by both methods was 382. From the table it is seen that the customary

TABLE 2

COMPARISON OF TITRES OBTAINED BY ORDINARY BACTERIAL AGGLUTINATION AND BY CENTRIFUGE TESTS.

	No. of sera	Difference in favour of centrifuge test						Diff. in favour of ordinary bacterial agglutination		Titre lower than 1:20 by both methods
		No. difference	1 tube	2 tubes	3 tubes	4 tubes	5 tubes	1 tube	2 tubes	
Titrations with homologous rabbit immune sera										
O titre	99	8	16	29	21	13	12	—	—	—
K titre	99	23	31	23	14	5	—	1	2	—
Titrations with patients' sera										
O titre	92	1	8	14	16	7	1	—	—	45
K titre	92	—	6	—	—	1	—	—	—	85
Total		32	61	66	51	26	13	1	2	130

bacterial agglutination test gave a higher titre than the centrifuge test in only three titrations. Even in these the differences were insignificant, one tube in one case and two tubes in two cases. Both methods yielded the same result in 32 titrations. When a difference of one tube is disregarded, the centrifuge method was better (difference two tubes or more) in 156 titrations. A difference of at least three tubes, which may be considered a significant difference, in favour of the centrifuge method was found in 90 titrations (23.6 ± 2.1 %). The largest difference in favour of the centrifuge method was 5 tubes; this difference was obtained in 13 titrations. The centrifuge method thus proved to be clearly more sensitive in the antibody titrations.

Haemagglutination tests. Demonstration of Escherichia coli antibodies by means of the haemagglutination test was performed using the following modification of the procedure of Neter et alii (1953). Human O red blood cells were washed three times with physiological saline and, after adding boiled homologous E. coli meat broth culture, incubated two hours at 37°. The modified blood cells were then washed three times with physiological saline. A two-fold dilution series was prepared from the serum of each patient starting from the 1:10 dilution and using 0.2-ml volumes. Two-tenths of a millilitre of the 2 per cent suspension of the modified red cells was added to each tube and the tubes incubated two hours in a water bath at 37° before the readings were made.

The strains used in the antibody determinations had been isolated from the appendix or peritoneal exudate of the patient in question and their antigen structures had been established with certainty. In every case control titrations were performed using the homologous rabbit serum. In addition, the serum of each patient was also tested with an O 111 strain (D 433) which had been chosen as the control strain.

HISTOPATHOLOGICAL CLASSIFICATION OF APPENDICES

For the histopathological examinations, specimens were taken from the macroscopically most inflamed regions of the appendices. Whenever an obstruction was seen to divide the appendix into a macroscopically clearly inflamed distal, and a slightly inflamed or uninflamed proximal region, specimens were taken from both regions.

The histopathological examinations were based on the criteria proposed by Büchner (1955).

Acute Focal Appendicitis. — Defective epithelium in the rugations of the appendical mucosa which contains leucocyte masses and fibrin.

A leucocyte mass is found below the eroded layer which expands like a wedge toward the serosa (Aschoff 1908).

Schenken et alii (1956), in a histological study of 2750 diseased appendices, found purulent exudate in the lumens of 901 appendices without observing any inflammatory changes in the walls of the appendices. They cut serial sections from 20 of these appendices and found a focal ulcer of the mucosa which was the source of the exudate in the lumen in 16. From this they concluded that in every appendix containing exudate in its lumen an epithelial defect should be found as a sign of impending appendicitis. This type of appendicitis they termed acute focal appendicitis. I have used the same name for appendices which have contained purulent exudate in their lumens or which have been characterized by an inflamed focus.

Acute Phlegmonous Appendicitis. — A diffuse leucocyte infiltration is observed in the mucosa and submucosa between the smooth muscle cells in the muscle layer, and in the subserosa. Serum from the capillaries has penetrated between the tissues and separated them.

Acute Ulcero-Phlegmonous Appendicitis. — A diffuse leucocyte infiltration permeates all layers of the appendix. In addition, large ulcers are seen in the mucosa.

Acute Abscessed Appendicitis. — The appendical wall is more or less permeated and damaged by numerous microscopic abscesses. The abscesses may extend from the lumen to the outside of the wall producing a microperforation.

Gangrenous Appendicitis. — Massive purulent accumulations that have developed from microabscesses in the appendix wall. The tissues are necrotic and the capillaries thrombotic.

Normal Appendix. — No changes of inflammatory origin are seen. The preparations were, as mentioned above, taken from the macroscopically most inflamed regions. The lumens of the appendices had been opened for taking specimens for bacteriological examination. This has been a disadvantage particularly from the standpoint of the histopathological classification of the appendices of the first group (Ac. Focal A.) since the region of possible primary erosion may have been destroyed and the exudate in the lumen may have been lost when the lumen was exposed.

MATERIAL

The specimens examined were collected at the Surgical Clinic of the University of Turku during the period from February, 1953, to September, 1955.

The following specimens were examined:

Appendices	312
Ileocoecal lymph nodes	113
Faecal specimens	278
Peritoneal exudates	72.

The following specimens were taken from various parts of the appendices:

Specimens from the lumens	310
Specimens from lumens proximal to obstructions	35
Wall tissue specimens	289.

In all 4089 colonies, including colonies that did not ferment lactose, were examined. Of the isolated strains, 3500 were identified as Escherichia coli strains. They are listed according to site of origin in Table 3.

TABLE 3

NUMBER OF SPECIMENS EXAMINED AND THE NUMBER OF E. COLI STRAINS ISOLATED

Source	Specimens	E. coli strains
Appendical lumen	310	1369
Proximal part of appendix	35	155
Wall tissue	289	611
Peritoneal exudate	72	175
Lymph node	113	66
Faeces	278	1124
Total	1097	3500

RESULTS

HISTOPATHOLOGICAL FINDINGS

On the basis of their histopathological appearance, the appendices were classified as shown in Table 4.

The uninflamed appendices form the control group. These were

TABLE 4

HISTOPATHOLOGICAL CLASSIFICATION OF EXAMINED APPENDICES.

Type of appendicitis	Number	Perforative
Acute focal A.	24	
Phlegmonous A.	82	
Ulcero-phlegmonous A.	45	
Abscessed A.	37	5
Gangrenous A.	69	35
Tuberculous A.	2	
Total in pathological material	259	40
Normal appendices	53	
Grand total	312	40

removed in connection with other abdominal operations for the purpose of this study. In a part of the patients, laparotomy had been performed because of suspected appendicitis, but some other cause, usually gynaecologic (ruptured or twisted ovarial cyst), had been found for the symptoms in the appendical region.

Tuberculous appendices were not taken into consideration when studying the occurrence of Escherichia coli since they represent a specific type of infection.

The largest group of pathological appendices were of the plegmonous type (31.9 ± 2.9 %), while gangrenous appendices formed the next largest group (26.8 ± 2.8 %). Perforation had occurred in 40 patients, in five patients with abcessed appendices and in thirty-five with gangrenous appendices.

BACTERIOLOGICAL RESULTS

Bacteria were found in the lumen specimens from all the inflamed appendices and only one of the uninflamed appendices was free of bacteria (Table 5).

Of the tissue specimens taken from the walls of the appendices of the appendicitis cases, 39.4 ± 3.3 per cent were not found to contain bacteria. The incidence of bacteria in the appendical wall

specimens increased with the severity of the inflammation. Of 142 wall specimens from patients with mildly inflamed appendices, groups acute focal, phlegmonous and ulcero-phlegmonous, 69 ($48.6 \pm 4.2\%$) yielded no bacteria on culture, whereas 26 (26.3 ± 4.4 %) of the 99 appendix wall specimens from patients suffering from severe forms of appendicitis, abscessed and gangrenous, were free of bacteria. None of the wall specimens from normal appendices yielded bacteria.

No bacterial growth resulted in 40.3 ± 6.0 per cent of the peritoneal exudate cultures. In all of the 40 cases where bacterial growth occurred, the peritoneal exudates were clearly purulent and all appendices were perforated. Five peritoneal exudate specimens from patients with no inflammatory changes in the appendices did not exhibit bacterial growth. In four of these patients the peritoneal exudate had originated in haemorrhagic corpora lutea, and in one patient, a ten-year-old boy, the origin was in hyperplastic mesenteric lymph nodes.

Cultures of 81.5 ± 3.6 per cent of the lymph node specimens were negative. This percentage is probably too high since the bacteria within small lymph nodes may have been destroyed by the immersion in 94 per cent alcohol and sterilization. It may be mentioned that four of the 28 lymph nodes that were removed from patients with uninflamed appendices contained bacteria.

An abundant bacterial growth resulted from all the faecal specimens taken, also from those of the, mainly peritonitis, patients who had been given antibiotics and sulphonamides before the specimens were taken.

Escherichia coli was isolated from lumen specimens from 94.2 ± 1.5 per cent of the inflamed appendices and from 86.5 ± 4.9 per cent of the uninflamed appendices. The difference between these two percentages is not statistically significant.* Of the specimens from the appendices proximal to lumen obstructions, 94.2 ± 4.1 per cent were found to contain E. coli. E. coli was isolated from 88.4 ± 2.7 per cent of the wall tissue specimens from inflamed appendices; this percentage does not differ significantly from that for the lumen specimens of the same material. The percentage for the peritoneal exudates was 97.5 ± 2.5. Sixteen of the

* Statistical analyses according to Alameri and Pöyhönen (1954).

TABLE 5

BACTERIAL GROWTH AND THE INCIDENCE OF ESCHERICHIA COLI.

Type of appendicitis	Lumen				Proximal part			Wall tissue			
	No. of cases	No growth	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth	No. of cases	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth	No. of cases	No growth	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth
Acute focal A.	24	—	24	24	1	1	1	21	15	6	6
Phlegmonous A.	82	—	82	75	5	5	5	80	37	43	33
Ulcero-phlegmonous A.	45	—	45	42	5	5	5	41	17	24	23
Abscessed A.	37	—	37	36	6	6	4	35	16	19	19
Gangrenous A.	69	—	69	65	17	17	17	64	10	54	48
Pathological material	257	—	257	242	34	34	32	241	95	146	129
Per cent				94.2 ± 1.5			94.2 ± 4.1		39.4 ± 3.3		88.4 ± 2.7
Normal appendices ..	53	1	52	45	1	1	1	48	48		
				86.5 ± 4.9							
Total	310	1	309	287	35	35	33	289	143	146	129
Per cent				92.5 ± 1.5			94.4 ± 3.9		49.5 ± 2.9		88.4 ± 2.7

cultures of 21 lymph nodes from which bacteria grew contained E. coli. E. coli was isolated from 94.8 ± 1.5 per cent of the faecal specimens from patients with diseased appendices and from 97.7 ± 2.2 per cent of the faecal specimens from patients with normal appendices.

SEROLOGICAL RESULTS

The O-groupable Escherichia Coli Strains. — The occurrence of E. coli strains in different types of appendicitis and different sites and the O groupability of the strains are shown in Table 6. The proportion of O-groupable strains among those isolated from the lumen specimens from inflamed appendices is 76.7 ± 1.3 per cent. No significant differences are noted between the various types of appendicitis in the O groupability of the luminal strains.

Peritoneal exudate				Lymph node				Faeces		
No. of cases	No growth	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth	No. of cases	No growth	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth	No. of cases	No. of cases with positive bacterial growth	No. of cases with positive E. coli growth
1	1	—	—	10	10	—	—	22	22	22
9	9	—	—	25	21	4	3	69	69	68
7	7	—	—	16	14	2	2	38	38	38
9	4	5	5	15	10	5	3	35	35	32
41	6	35	34	19	13	6	5	68	68	60
67	27	40	39	85	68	17	13	232	232	220
	40.3 ± 6.0		97.5 ± 2.5		80.0 ± 4.3		76.5 ± 10.0			94.8 ± 1.5
5	5			28	24	4	3	46	46	45
										97.7 ± 2.2
72	32	40	39	113	92	21	16	278	278	265
	43.1 ± 5.9		97.5 ± 2.5		81.5 ± 3.6		76.2 ± 9.3			95.3 ± 1.3

Of the strains isolated from lumen specimens from normal appendices, 57.0 ± 3.2 per cent could be entered into O groups 1—25. Of the strains from the proximal parts of the inflamed appendices, 80.0 ± 3.3 per cent were O-groupable, and of the strains from wall specimens 77.1 ± 1.7 per cent. The percentage of O-groupable strains among those isolated from peritoneal exudates was 88.0 ± 2.5 per cent and among those from the lymph nodes 65.2 ± 5.9 per cent. 49.5 ± 1.6 per cent of the faecal strains isolated from appendicitis patients and 41.8 ± 3.5 per cent of the faecal strains from patients with normal appendices were O-groupable.

From the above figures it is seen that the proportion of O-groupable strains in the specimens from pathological material is nearly twenty per cent higher than in the specimens from normal appendices. A similar difference is noted between the proportion

TABLE 6

OCCURRENCE OF E. COLI STRAINS OF O GROUPS 1—25 IN DIFFERENT TYPES OF INFLAMED APPENDICES.

Type of appendicitis	Lumen			Proximal part			Wall tissue			Peritoneal exudate			Lymph node			Faeces		
	No. of strains examined	O-groupable	Per cent	No. of strains examined	O-groupable	Per cent	No. of strains examined	O-groupable	Per cent	No. of strains examined	O-groupable	Per cent	No. of strains examined	O-groupable	Per cent	No. of strains examined	O-groupable	Per cent
Ac. foc. A.	122	89	73.0 ± 4.0	5	5		26	19	73.1 ± 8.8	—	—		—	—		95	47	49.5 ± 5.1
Phlegm. A.	356	285	80.1 ± 2.1	21	14		161	141	87.5 ± 2.6	—	—		15	10		298	158	53.0 ± 2.9
Ulc-phl. A.	195	146	74.9 ± 3.1	23	23		110	76	69.1 ± 4.6	—	—		10	10		169	80	47.3 ± 3.9
Absc. A.	158	119	75.4 ± 3.4	19	16		85	59	69.4 ± 4.6	21	20		11	5		139	57	41.0 ± 4.2
Gangr. A.	301	230	76.5 ± 2.4	82	62		229	177	77.2 ± 2.3	154	134		15	10		220	115	52.3 ± 3.4
Pathol. material	1 132	869	76.7 ± 1.3	150	120	80.0 ± 3.3	611	472	77.1 ± 1.7	175	154	88.0 ± 2.5	51	35	68.7 ± 6.5	921	457	49.5 ± 1.65
Normal appendices	237	135	57.0 ± 3.2	5	5		—	—		—	—		15	8		203	85	41.8 ± 3.5
Total	1 369	1 004	73.3 ± 1.2	155	125	80.0 ± 3.3	611	472	77.1 ± 1.7	175	154	88.0 ± 2.5	66	43	65.2 ± 5.9	1 124	542	48.3 ± 1.5

of O-groupable strains among the strains from lumen specimens from diseased appendices and the proportion of such strains in the faecal specimens from patients with normal and inflamed appendices. The observed differences are highly significant. On the other hand, the O groupability is the same for the lumen and wall strains from diseased appendices. A significant difference in O groupability exists between the luminal and faecal strains isolated from normal appendices.

Of the 3,500 Escherichia coli strains isolated from all specimens, 2,340 or 66.9 ± 0.79 per cent could be entered into O groups 1—25 (Table 7).

TABLE 7

O-GROUPABLE STRAINS IN THE WHOLE MATERIAL.

	Specimens	Specimens containing E. coli	No. of E. coli strains examined	O-groupable strains	Per cent
Lumen	310	287	1 369	1 004	73.3 ± 1.2
Proximal part	35	33	155	125	80.0 ± 3.3
Wall tissue	289	129	611	472	77.1 ± 1.7
Peritoneal exudate	72	39	175	154	88.0 ± 2.5
Lymph node	113	16	66	43	65.2 ± 5.9
Faeces	278	265	1 124	542	48.3 ± 1.5
Total	1 097	769	3 500	2 340	66.9 ± 0.79

The Incidence of Different O Groups. — The incidence of Escherichia coli strains of O groups 1—25 in the different specimens from normal and pathological material is shown in Table 8. The incidence in specimens from the proximal parts of normal appendices and lymph nodes in these cases is not given owing to the small number of specimens examined.

E. coli strains of O groups 14 and 24 were not found in the examined material and only one strain of O group 17 was isolated from a faecal specimen of an appendicitis patient.

TABLE 8

THE OCCURRENCE OF DIFFERENT O GROUPS IN NORMAL AND PATHOLOGICAL MATERIAL GROUPED ACCORDING TO THE REGION FROM WHICH THE SPECIMEN WAS TAKEN.

L, lumen; Pr, proximal part; W, wall tissue; P, peritoneal exudate; LN, lymph node; F, faeces.

O group	Appendicitis (the whole series)						Normal appendices	
	L	Pr	W	P	LN	F	L	F
1	39	10	22	6	3	42	11	7
2	27	2	9	7	—	30	4	5
3	2	—	1	—	—	5	1	—
4	14	1	4	2	—	11	—	2
5	3	1	2	1	—	2	—	—
6	13	1	3	2	—	4	6	4
7	10	—	5	2	—	5	1	1
8	20	1	8	2	—	8	2	2
9	23	6	18	3	1	14	6	4
10	10	3	5	2	1	6	—	—
11	14	1	6	1	1	1	2	1
12	2	—	1	1	—	5	2	3
13	2	—	1	1	—	5	1	1
14	—	—	—	—	—	—	—	—
15	30	3	16	4	—	12	4	5
16	7	—	3	1	—	8	2	1
17	—	—	—	—	—	1	—	—
18	3	1	1	—	1	4	1	2
19	1	—	1	—	—	4	1	1
20	—	1	—	—	—	2	—	—
21	18	2	14	2	1	19	2	2
22	1	—	—	—	—	1	—	—
23	6	1	3	1	—	7	—	1
24	—	—	—	—	—	—	—	—
25	2	—	2	—	—	6	1	1
Cases with O-groupable E. coli strains	202	27	110	34	7	156	36	31

In order to determine whether strains of one O group are more prevalent than strains of other O groups in pathological and normal material or in some sampling site, the relative incidences of strains of the nine most common O groups were calculated. In Table 9 the O groups are listed in the order of their frequency in the lumen samples from the inflamed appendices, viz., O groups

TABLE 9

THE FREQUENCY OF OCCURRENCE OF VARIOUS O GROUPS IN DIFFERENT SPECIMENS FROM APPENDICITIS PATIENTS AND CONTROLS.

O group	Pathological material						Normal material			
	Lumen.		Wall tissue		Faeces		Lumen		Faeces	
	202 cases		110 cases		156 cases		36 cases		31 cases	
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
1	39	19.3 ± 2.8	22	20.0 ± 3.8	42	26.3 ± 3.6	11	30.5 ± 7.8	7	22.6 ± 7.4
15	30	14.8 ± 2.5	16	14.5 ± 3.4	12	7.7 ± 2.1	4	11.1 ± 5.3	5	16.1 ± 6.7
2	27	13.4 ± 2.4	9	8.2 ± 2.6	30	19.2 ± 3.2	4	11.1 ± 5.3	5	16.1 ± 6.7
9	23	11.4 ± 2.2	18	16.4 ± 3.5	14	9.0 ± 2.3	6	16.7 ± 6.2	4	12.9 ± 6.1
8	20	9.9 ± 2.1	8	7.3 ± 2.5	8	5.1 ± 1.8	2	(5.5)	2	(6.5)
21	18	8.8 ± 2.0	14	12.7 ± 3.2	19	12.2 ± 2.6	2	(5.5)	2	(6.5)
4	14	6.9 ± 1.8	4	3.6 ± 1.8	11	7.1 ± 2.1	—	—	2	(6.5)
11	14	6.9 ± 1.8	6	5.5 ± 2.2	1	(0.6)	2	(5.5)	1	(3.2)
6	13	6.4 ± 1.7	3	2.7 ± 1.6	4	2.6 ± 1.3	6	16.7 ± 6.2	4	12.9 ± 6.1

1, 15, 2, 9, 8, 21, 4, 11 and 6. Strains of the most common O group 1 were isolated from 19.3 ± 2.8 per cent of the lumen specimens that contained O-groupable E. coli strains. Strains of O group 6, which comprised the smallest group, were isolated from 6.4 ± 1.7 per cent of the lumen specimens containing O-groupable strains. The frequencies for the strains of the other O groups were fairly evenly distributed between these two percentages and it cannot be concluded that any particular group predominated over the others. Likewise no O group clearly predominated in the wall specimens, in the lumen specimens from normal appendices, or in either of the two groups of faecal specimens. A comparison of the incidence of O groups in the lumen and faecal specimens of patients with diseased appendices shows that the incidence of strains of an O group did not differ significantly in the different groups of specimens.

O inagglutinability. — When the incidence of O-inagglutinable E. coli strains, i.e. the existence of capsular forms, among the strains isolated from the various types of appendicitis is examined (Table 10), it is found that no differences are noted even in this respect. In the whole material 92.1 ± 0.9 per cent of the lumen strains, 91.7 ± 1.2 per cent the wall strains, 92.3 ± 2.1 per cent of the peritoneal exudate strains, and 92.0 ± 2.4 per cent of the strains from the proximal parts were O-inagglutinable. The proportion of strains from the lumens of normal appendices that were O-inagglutinable was 88.9 ± 2.7 per cent. The difference in O inagglutinability in the pathological (93.7 ± 0.8 %) and the normal lumen material is not significant. 84.8 ± 1.7 per cent of the faecal strains from patients with diseased appendices were O-inagglutinable; this percentage differs significantly from that for the strains from lumen specimens of the same patient group. The percentage of O-inagglutinable strains among the strains isolated from faecal specimens of normal subjects was 84.7 ± 3.9 per cent. Of the O-groupable strains isolated from the lymph nodes 81.5 ± 5.9 per cent were O-inagglutinable.

The Incidence of Strains Possessing a K Antigen. — It was seen above that no O group predominated over the others in either the pathological or normal material. It has, however,

TABLE 10

O INAGGLUTINABILITY.

Type of appendicitis	Lumen			Prox. part			Wall tissue			Peritoneal exudate			Lymph node			Faeces		
	O-groupable strains	O-inagglutinable strains	Per cent	O-groupable strains	O-inagglutinable strains	Per cent	O-groupable strains	O-inagglutinable strains	Per cent	O-groupable strains	O-inagglutinable strains	Per cent	O-groupable strains	O-inagglutinable strains	Per cent	O-groupable strains	O-inagglutinable strains	Per cent
Ac. foc. A.	89	81	91.0 ± 3.1	5	5		19	14	73.7 ± 10.8	—	—		—	—		47	46	97.8 ± 2.2
Phlegm. A.	285	255	89.5 ± 1.9	14	14		141	118	83.8 ± 3.1	—	—		10	5		158	133	84.2 ± 2.9
Ulc-phl. A.	146	138	94.5 ± 1.9	23	21		76	73	96.0 ± 2.2	—	—		10	10		80	63	78.8 ± 4.6
Absc. A	119	109	91.5 ± 2.5	16	16		59	57	96.7 ± 2.3	20	20		5	5		57	42	73.6 ± 5.9
Gangr. A.	230	222	96.5 ± 1.2	62	54		177	171	96.6 ± 1.4	134	123		10	10		115	104	90.5 ± 2.7
Pathol. material	869	805	93.7 ± 0.8	120	110	91.7 ± 2.5	472	433	91.7 ± 1.2	154	143	92.3 ± 2.1	35	30	85.7 ± 6.0	457	388	84.8 ± 1.7
Normal appendices	135	120	88.9 ± 2.7	5	5		—	—		—	—	—	—	8	5		85	72	84.7 ± 3.9
Total	1004	925	92.1 ± 0.9	125	115	92.0 ± 2.4	472	433	91.7 ± 1.2	154	143	92.3 ± 2.1	43	35	81.5 ± 5.9	542	460	85.0 ± 1.5

TABLE 11

INCIDENCE OF MOST COMMON K ANTIGENS IN EXAMINED SPECIMENS.

K-antigen	Appendicitis				Faeces of appendicitis patients		Normal appendices		Faeces of patients with normal appendices	
	Lumen		Wall tissue							
	216 specimens		114 specimens		185 specimens		40 specimens		38 specimens	
	Specimens	%	Specimens	%	Specimens	%	Specimens	%	Specimens	%
1 L	55	25.4 ± 3.1	21	18.4 ± 3.7	61	33.0 ± 3.5	9	22.5 ± 6.6	11	28.9 ± 7.4
5 L	35	16.2 ± 2.6	15	13.2 ± 3.2	21	11.4 ± 2.3	9	22.5 ± 6.6	3	7.9 ± 4.5
20 L	21	9.2 ± 2.1	13	11.4 ± 3.0	13	7.0 ± 1.9	1	(2.5)	1	(2.6)
14 L	16	7.4 ± 1.8	13	11.4 ± 3.0	10	5.4 ± 1.7	1	(2.5)	2	(5.3)
51 L	15	6.9 ± 1.7	8	7.0 ± 2.4	5	2.7 ± 1.2	5	12.5 ± 5.2	2	(5.3)
10 L	10	4.6 ± 1.4	5	4.4 ± 1.9	3	1.6 ± 0.9	1	(2.5)	—	—

been stated in the literature that capsular strains are in general more pathogenic than acapsular strains. On this basis it might be possible that a certain K antigen may be found in the most pathogenic strains and that strains possessing this antigen may be more common in pathological than in normal material. This was not, however, found to be the case in the present series as shown in Table 11, where the incidence of the six most frequently found K antigens is given. The K antigen most often found in the pathological and normal specimens was the 1 L antigen but its frequency did not differ significantly in the two groups of specimens. The other common K antigens were 5L, 20L, 14L, 51L and 10L, and their frequencies in the pathological and normal materials did not differ to such an extent that any conclusions can be drawn. For example, the most common 1L and 5L antigens were found in 90 (41.8 ± 3.4 %) of the 216 lumen specimens from inflamed appendices and in 36 (31.6 ± 4.4 %) of 114 wall specimens from inflamed appendices. On the other hand, these K antigens were found in 18 (45.0 ± 8.0 %) of the 40 lumen specimens from normal appendices and in 82 (44.3 ± 3.7 %) of 185 faecal specimens of appendicitis patients.

The Incidence of Strains Possessing an H Antigen. — Of the isolated 3,500 Escherichia coli strains 2,384 (68.1 ± 0.8 %) were motile. Some of the motility tests were performed only after the strains had been stored several months, in some cases even one year, on egg medium. This may have led to a loss of motility, and hence it is possible that if the motility tests had been performed immediately after the strains had been isolated, the proportion of motile strains might have been higher.

The presence of H antigens was studied using H immune sera 1—33. The number of motile strains among the 2297 (Table 12) whose antigenic structures were determined was 1416. The H antigen could be identified in 1203 (85.0 ± 0.9 %) of these.

The Antigenic Structures of the Escherichia Coli Strains. — The antigenic structures of the Escherichia coli strains isolated from all specimens except the lymph nodes are given in Table 12. In this table antigenic structures are designated as proposed by Kauffmann, Ørskov and Ewing (1956). Since no E. coli serotype

TABLE 12

INCIDENCE OF E. COLI SEROTYPES IN HEALTHY AND INFLAMED APPENDICES AND IN FAECES SPECIMENS.

The serotypes printed in bold-face are found in the antigenic schema. The figures in brackets denote the total number of specimens examined.

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O1											
O1:K 1(L):H7	19	75	4	7	3	4	18	52		138	11
O1:K 1(L):—	22	83	2	2	1	4	16	37		126	13
O1:K 1(L):H2	—	—	—	—	—	—	1	1		1	
O1:K 1(L):H4	1	1	—	—	—	—	—	—		1	
O1:K 5(L):H?	—	—	1	2	—	—	1	1		3	1
O1:K51(L):H7	14	64	4	16	2	2	5	13		95	4
O1:K51(L):—	5	22	1	2	—	—	—	—		24	
O1: — :—	4	10	—	—	—	—	2	6		16	
O1: — :H?	—	—	—	—	1	1	3	7		8	
O1:K ? :H?	—	—	—	—	—	—	1	1		1	
	(49)	255	(11)	29	(7)	11	(42)	118	(109)	413	29
O2											
O2:K 1(L):H4	4	12	1	1	—	—	1	3		16	1
O2:K 1(L):—	6	30	1	3	2	8	8	16		57	1
O2:K 1(L):H7	5	22	—	—	1	4	1	2		28	4
O2:K2a2b(L):H1	2	14	—	—	—	—	—	—		14	12
O2:K 1(L):H?	1	1	—	—	—	—	7	12		13	
O2:K 5(L):H4	12	55	1	1	—	—	7	10		66	47
O2:K 5(L):—	7	31	1	1	—	—	4	10		42	3
O2:K15(L):H1	1	5	—	—	1	1	1	5		11	10
O2:K51(L):H7	—	—	—	—	1	1	—	—		1	
O2: — :—	—	—	—	—	—	—	3	6		6	2
O2: — :H?	1	6	1	1	—	—	3	3		10	6
O2:K ? :H?	—	—	—	—	—	—	1	1		1	
	(33)	176	(4)	7	(5)	14	(30)	68	(72)	265	86
O3											
O3:K2a2b(L):H2	1	5	1	4	—	—	1	1		10	10
O3: — :H?	2	10	—	—	—	—	3	4		14	
O3:K ? :H?	—	—	—	—	—	—	1	3		3	
	(2)	15	(1)	4	—	—	(5)	8	(8)	27	10
O4											
O4:K 3(L):H5	1	4	—	—	—	—	—	—		4	4
O4:K 3(L):—	3	19	—	—	—	—	2	3		22	22

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O4:K 6(L):H5	—	—	—	—	—	—	1	1		1	1
O4:K 6(L):—	1	1	—	—	—	—	—	—		1	
O4:K 9(L):H?	—	—	—	—	—	—	1	1		1	
O4:K12(L):H5	4	14	—	—	1	2	1	1		17	17
O4:K12(L):—	2	8	—	—	—	—	—	—		8	8
O4:K52(L):H4	2	2	—	—	—	—	1	1		3	
O4:K52(L):—	3	12	—	—	1	1	2	3		16	
O4: — :H5	2	14	—	—	—	—	—	—		14	14
O4:K ? :H?	—	—	—	—	—	—	2	6		6	
O4: — :H?	—	—	—	—	—	—	1	2		2	
	(15)	74	—	—	(2)	3	(11)	18	(28)	95	66
O5											
O5:K 4(L):H4	—	—	—	—	—	—	1	1		1	
O5:K 3(L):H?	2	8	—	—	—	—	—	—		8	
O5:K 7(L):—	—	—	—	—	—	—	1	3		3	
O5:K ? :—	1	1	—	—	—	—	—	—		1	
O5: — :H?	1	3	—	—	—	—	—	—		3	
	(4)	12	—	—	—	—	(2)	4	(6)	16	
O6											
O6:K 1(L):H?	1	5	—	—	—	—	1	5		10	
O6:K2a2c(L):H1	1	5	—	—	—	—	—	—		5	
O6:K 5(L):H1	—	—	1	5	—	—	—	—		5	
O6:K 5(L):—	2	16	—	—	—	—	—	—		16	16
O6:K 5(L):H?	4	18	—	—	—	—	—	—		18	14
O6:K13(L):H1	3	12	3	13	3	7	2	6		38	13
O6:K53(L):H33	1	2	—	—	—	—	—	—		2	2
O6:K53(L):H?	—	—	1	3	1	3	—	—		6	6
O6: — :H1	1	5	1	5	—	—	—	—		10	5
O6: :H?	3	14	—	—	—	—	—	—		14	10
O6:K ? :—	1	5	—	—	—	—	1	2		7	4
	(15)	82	(6)	26	(4)	10	(4)	13	(29)	131	66
O7											
O7:K 1(L):—	8	38	—	—	—	—	2	2		40	
O7:K 1(L):H?	1	2	—	—	—	—	—	—		2	
O7:K 7(L):H4	4	10	—	—	—	—	2	4		14	
O7: — :H4	1	8	1	3	1	1	1	2		14	

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O7: — :H?	1	1	—	—	—	—	—	—		1	
O7:K ? :H4	1	1	—	—	—	—	—	—		1	
O8	(14)	60	(1)	3	(1)	1	(5)	8	(21)	72	
O8:K25(B):H9	1	1	—	—	—	—	1	1		2	
O8:K25(B):—	1	1	—	—	—	—	1	1		2	
O8:K27(A):H10	1	10	—	—	—	—	1	1		11	
O8:K28(A):H19	2	10	—	—	1	1	—	—		11	
O8:K32(A):H27	1	4	—	—	—	—	—	—		4	
O8:K40(A):H9	1	10	—	—	—	—	1	1		11	
O8:K41(A):H?	1	2	—	—	—	—	—	—		2	
O8:K42(A):H4	4	23	—	—	—	—	1	3		26	
O8:K42(A):—	—	—	1	3	—	—	—	—		3	
O8:K43(A):H11	1	10	1	1	—	—	—	—		11	
O8:K43(A):—	1	4	—	—	—	—	1	3		7	7
O8:K44(A):H?	1	2	—	—	—	—	—	—		2	
O8:K44(A):—	1	3	—	—	—	—	—	—		3	
O8:K46(A):H4	—	—	—	—	1	3	—	—		3	
O8:K48(A):H9	2	7	—	—	—	—	—	—		7	
O8:K49(A):H11	1	1	—	—	—	—	1	1		2	
O8:K50(A):—	3	17	—	—	—	—	1	2		19	
O8:K50(A):H19	1	1	—	—	—	—	—	—		1	
O8: — :—	1	1	—	—	—	—	—	—		1	
O8:K ? :H?	1	1	—	—	—	—	—	—		1	
O9	(23)	108	(2)	4	(2)	4	(8)	13	(35)	129	7
O9:K26(A):H19	1	1	—	—	—	—	—	—		1	
O9:K28(A):H?	1	1	—	—	—	—	—	—		1	
O9:K29(A):—	1	9	—	—	—	—	1	1		10	
O9:K30(A):H19	3	28	—	—	2	2	—	—		30	3
O9:K30(A):—	1	1	—	—	—	—	1	1		2	
O9:K31(A):H4	3	11	—	—	—	—	1	1		12	
O9:K31(A):—	4	11	—	—	—	—	3	8		19	
O9:K32(A):—	2	15	1	3	—	—	1	5		23	3
O9:K32(A):H25	—	—	—	—	—	—	1	1		1	
O9:K33(A):—	5	25	4	16	—	—	2	7		48	2
O9:K33(A):H?	3	9	—	—	—	—	1	1		10	
O9:K34(A):H4	1	5	—	—	—	—	—	—		5	

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O9:K34(A):—	2	16	—	—	—	—	3	7		23	
O9:K35(A):—	2	3	—	—	—	—	—	—		3	
O9:K36(A):H19	4	22	—	—	—	—	—	—		22	
O9:K36(A):—	2	7	—	—	—	—	—	—		7	
O9:K37(A):—	5	20	1	1	—	—	2	8		29	
O9:K37(A):H?	1	2	1	1	—	—	—	—		3	
O9:K55(A):H4	—	—	—	—	1	1	—	—		1	
O9:K55(A):—	—	—	—	—	—	—	1	4		4	
O9: — :—	1	1	—	—	—	—	—	—		1	
O9:K ? :—	2	3	—	—	1	1	—	—		4	
O9:K ? :H?	1	2	—	—	—	—	—	—		2	
O10	(33)	192	(6)	21	(4)	4	(14)	44	(57)	261	8
O10:K 5(L):H4	7	18	—	—	—	—	1	1		19	4
O10:K 5(L):—	10	48	—	—	—	—	1	2		50	
O10:K 7(L):H?	1	4	—	—	—	—	1	3		7	
O10: — :H4	1	3	—	—	—	—	1	2		5	
O10: — :—	1	2	—	—	—	—	1	1		3	
O10:K ? :H4	—	—	—	—	—	—	1	1		1	
O11	(13)	75	—	—	—	—	(6)	10	(19)	85	4
O11:K 4(L):H4	1	5	—	—	—	—	—	—		5	
O11:K10(L):H10	9	32	1	2	—	—	—	—		34	
O11:K10(L):—	5	38	—	—	—	—	—	—		38	
O11:K52(L):H4	1	3	1	5	—	—	—	—		8	
O11:K52(L):—	—	—	—	—	—	—	1	5		5	
O11: — :H4	2	8	—	—	1	3	—	—		11	
O11:K ? :H4	1	4	—	—	—	—	—	—		4	
O12	(16)	90	(2)	7	(1)	3	(1)	5	(20)	105	
O12:K 1(L):H4	2	14	—	—	1	1	1	1		16	
O12:K 1(L):—	1	1	—	—	1	2	—	—		3	
O12:K 5(L):H4	1	1	1	4	—	—	1	3		8	
O12:K 5(L):—	1	1	1	1	1	2	1	2		5	
O12:K24(L):H30	—	—	—	—	—	—	1	3		3	
O12: — :H?	—	—	1	1	—	—	—	—		1	
O12:K ? :H4	—	—	—	—	1	1	1	1		2	
	(3)	17	(2)	6	(3)	6	(5)	10	(13)	39	

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O13											
O13:K 5(L):H1	2	14	—	—	—	—	—	—		14	14
O13:K11(L):H11	—	—	—	—	1	2	3	4		6	
O13:K11(L):—	—	—	—	—	—	—	1	1		1	
O13: — :H11	—	—	1	1	—	—	1	1		2	
	(2)	14	(1)	1	(1)	2	(5)	6	(9)	23	14
O14	—	—	—	—	—	—	—	—		—	
O15											
O15:K 1(L):H17	1	1	—	—	—	—	—	—		1	
O15:K 7(L):H33	3	25	—	—	—	—	—	—		25	3
O15:K14(L):H4	7	27	1	5	2	2	1	2		36	7
O15:K14(L):—	6	34	—	—	1	3	—	—		37	
O15:K14(L):H32	1	3	—	—	—	—	—	—		3	
O15:K20(L):H4	10	39	—	—	—	—	4	9		48	10
O15:K20(L):—	11	40	1	3	1	3	2	2		48	7
O15:K ? :H27	2	7	—	—	—	—	—	—		7	
O15: — :—	2	5	—	—	—	—	2	2		7	
O15: — :H4	1	7	—	—	—	—	—	—		7	
O15: — :H?	—	—	1	2	2	5	4	6		13	
O15:K ? :—	2	3	—	—	—	—	—	—		3	
O15:K ? :H?	1	9	1	1	—	—	1	1		11	
	(36)	200	(4)	11	(5)	13	(12)	22	(57)	245	27
O16											
O16:K 1(L):—	4	13	2	5	1	1	4	15		34	
O16:K 1(L):H2	1	2	—	—	—	—	—	—		2	
O16:K 1(L):H4	—	—	—	—	—	—	1	1		1	
O16:K 1(L):H28	—	—	—	—	—	—	1	2		2	
O16:K14(L):H4	1	5	—	—	—	—	—	—		5	
O16:K20(L):H4	1	2	—	—	—	—	—	—		2	
O16:K ? :H4	2	2	—	—	—	—	—	—		2	
O16: — :H?	—	—	—	—	—	—	2	2		2	
	(9)	24	(2)	5	(1)	1	(8)	20	(20)	50	
O17											
O17:K ? :—	—	—	—	—	—	—	1	1		1	
	—	—	—	—	—	—	(1)	1	(1)	1	

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains	
O18											
O18:K 1(L):H7	1	5	—	—	—	—	—	—		5	
O18:K 5(L):H4	2	18	—	—	—	—	2	2		20	
O18:K 5(L):—	—	—	1	2	1	2	—	—		4	
O18: — :7	—	—	—	—	—	—	2	2		2	
O18: — :H14	—	—	—	—	1	1	—	—		1	
	(3)	23	(1)	2	(2)	3	(4)	4	(10)	32	
O19											
O19:K 5(L):H4	2	10	—	—	—	—	1	2		12	
O19: — :H1	—	—	—	—	1	1	—	—		1	
O19: — :H?	—	—	1	1	—	—	3	5		6	
	(2)	10	(1)	1	(1)	1	(4)	7	(8)	19	
O20											
O20:K14(L):—	1	1	—	—	—	—	—	—		1	
O20:K17(L):—	—	—	—	—	—	—	1	1		1	
O20:K ? :—	—	—	—	—	—	—	1	1		1	
	(1)	1	—	—	—	—	(2)	2	(3)	3	
O21											
O21:K 1(L):H4	2	15	—	—	—	—	—	—		15	
O21:K 4(L):H4	1	10	—	—	—	—	—	—		10	
O21:K10(L):—	—	—	—	—	—	—	1	1		1	
O21:K14(L):H4	10	43	—	—	—	—	5	21		64	
O21:K14(L):H9	—	—	—	—	—	—	1	1		1	
O21:K20(L):H4	5	17	—	—	—	—	7	15		32	
O21:K20(L):—	5	10	—	—	—	—	3	4		14	
O21: — :H4	—	—	—	—	—	—	1	5		5	
O21: — :—	—	—	—	—	—	—	1	1		1	
O21: — :H?	2	2	—	—	1	1	2	2		5	
O21:K ? :H4	6	27	2	9	1	4	1	4		44	
O21:K ? :—	3	6	—	—	—	—	—	—		6	
	(25)	130	(2)	9	(2)	5	(19)	54	(48)	198	
O22											
O22:K 6(L):H?	1	1	—	—	—	—	1	1		2	2
O22:K 6(L):—	1	4	—	—	—	—	1	4		8	8
	(1)	5	—	—	—	—	(1)	5	(2)	10	10
O23											
O23:K 1(L):—	1	4	—	—	—	—	—	—		4	
O23:K 5(L):H1	2	8	—	—	—	—	1	3		11	11

Serotype	Pathological material		Normal appendices		Faeces of patients with healthy appendices		Faeces of appendicitis patients		Total		No. of haemolytic strains
	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	No. of strains	No. of specimens	Strains total	
O23:K 5(L):—	—	—	—	—	1	3	—	—		3	
O23:K14(L):H6	—	—	—	—	—	—	1	2		2	
O23:K18(L):H15	—	—	—	—	—	—	1	1		1	
O23:K21(L):H15	—	—	—	—	—	—	1	1		1	
O23:K21(L):H?	1	2	—	—	—	—	2	3		5	
O23:K24(L):H?	1	7	—	—	—	—	—	—		7	
O23:K24(L):—	1	1	—	—	—	—	—	—		1	
O23: — :—	1	2	—	—	—	—	—	—		2	
O23: — :H15	2	15	—	—	—	—	—	—		15	
O23: — :H?	—	—	—	—	—	—	1	1		1	
	(7)	39	—	—	(1)	3	(7)	11	(15)	53	11
O24	—	—	—	—	—	—	—	—		—	
O25											
O25:K19(L):H12	2	10	—	—	—	—	1	1		11	6
O25: — :H9	1	2	—	—	—	—	—	—		2	
O25: — :H?	1	1	—	—	1	1	2	2		4	
O25: — :—	—	—	—	—	—	—	2	2		2	
O25:K ? :H?	—	—	1	4	—	—	1	1		5	
	(3)	13	(1)	4	(1)	1	(6)	6	(11)	24	6
Strains total	1.615		140		85		457		2.297		344

predominated over the others in the various types of appendicitis or in the peritoneal exudates, all the strains from pathological processes are presented as a single group in the table. The Escherichia coli strains that were isolated from normal appendices, from faecal specimens of patients with normal appendices and from faecal specimens of appendicitis patients have been considered as three separate groups.

All combinations of O, K and H antigens that were encountered are included in the table. In addition, a number of strains

whose K or H antigens or both could not be identified during the course of as many as three separate examinations are given.

Of the 2,297 strains in the table for which serological structures were determined, 1,226 strains could be classified according to the antigenic schema of Kauffmann—Knipschildt—Vahlne. Also the structures of 30 of the 43 strains from lymph node specimens whose serological structures were elucidated conformed with the antigenic schema. Thus the serotype could be determined for 2,340 of the 3,500 Escherichia coli strains and of these 1,256 or 56.7 ± 1.2 per cent could be classified according to the antigenic schema.

It was already noted that no O group was more prevalent than the others. Likewise no definite serotype was found in significantly higher incidence than the others in the inflamed appendices, in the normal appendices or in the faecal specimens.

Haemolytic Strains. — The number of haemolytic strains among the 2,297 serologically classified Escherichia coli strains was 344 or 15.0 ± 0.7 per cent (Table 12). The number of haemolytic strains among the typed 1,615 strains of pathological origin was 261, or 16.2 ± 0.9 per cent. Haemolytic strains comprised 12.1 ± 2.8 per cent of the 140 strains from normal appendices, 13.0 ± 3.7 per cent of the 85 faecal strains of patients with healthy appendices, and 12.0 ± 1.5 per cent of the 457 faecal strains of appendicitis patients. A comparison of the percentages shows that no significant differences exist in the incidence of haemolytic strains in the various groups.

Incidence of One or More Serotypes in the Same Specimen. — The number of specimens in which only one and in which two or more serotypes defined by O, K and H antigens were found are given in Table 13.

All the strains isolated from 48.4 ± 3.2 per cent of the lumen specimens from diseased appendices were of the same serotype in each case, while the corresponding percentage of the lumen specimens from healthy appendices was 35.6 ± 7.1 per cent. The difference between the percentages is not significant. The strains isolated from 57.4 ± 4.4 per cent of the wall specimens and from 61.6 ± 7.8 per cent of the peritoneal exudates had the same

TABLE 13

PROPORTIONS OF SPECIMENS IN WHICH ALL EXAMINED STRAINS WERE ALL OF ONE OR OF SEVERAL SEROTYPES.

Type of appendicitis	Lumen				Wall tissue				Peritoneal exudate				Faeces			
	Cases with E. coli	One serotype	Per cent	Two or more serotypes	Cases with E. coli	One serotype	Per cent	Two or more serotypes	Cases with E. coli	One serotype	Per cent	Two or more serotypes	Cases with E. coli	One serotype	Per cent	Two or more serotypes
Ac. foc. A.	24	9	37	15	6	2		4	—	—		—	22	2	9	20
Phlegm. A.	75	39	52	36	33	20	57	13	—	—		—	68	10	15	58
Ulc-phl. A.	42	16	38	26	23	10	43	13	—	—		—	38	7	18	31
Absc. A.	36	19	52	17	19	10	55	9	5	3		2	32	5	16	27
Gangr. A.	65	35	53	30	48	32	64	16	34	21		13	60	11	18	49
Pathol. material	242	118	48.4 ± 3.2	124	129	74	57.4 ± 4.4	55	39	24	61.6 ± 7.8	15	220	35	15.9 ± 2.8	185
Normal appendices	45	16	35.6 ± 7.1	29	—	—		—	—	—		—	45	5	11.1 ± 4.7	40
Total	287	134	46.8 ± 2.9	153	129	74	57.4 ± 4.4	55	39	24	61.6 ± 7.8	15	265	40	15.1 ± 2.6	225

antigenic structures. The differences between these percentages and the percentage for the pathological lumen material are not significant. The strains isolated from 15.9 ± 2.8 per cent of the faecal specimens from appendicitis patients and from 11.1 ± 4.7 per cent of the faecal specimens from patients with uninflamed appendices were of the same serotype. The differences between these two percentages and the percentage for the pathological specimens are highly significant, as is also the difference between the percentages for the lumen and faecal specimens from patients with normal appendices.

It is thus seen that the E. coli florae in lumens and walls of inflamed appendices and in peritoneal exudates are serologically much more uniform than the faecal E. coli florae, i.e. a greater number of serotypes are found in faecal than in appendical specimens.

The Incidence of the Same Escherichia Coli Serotype in Different Parts of the Appendix, in the Peritoneal Exudate and in the Faeces. — The numbers of cases in which the same E. coli serotype was isolated from different specimens from the same patient are given in Table 14. The specimens are compared in pairs, the lumen specimens with the specimens from the proximal parts, the wall specimens with the lumen specimens, the peritonael exudates with the lumen specimens, the lumen specimens with the faecal specimens, and the peritoneal exudates with the faecal specimens. The purpose of the comparison was to determine whether the same E. coli serotype is transferred from the faeces through the proximal part to the lumen of the appendix and further into the wall and the peritoneal cavity. Only such cases have been considered comparable in which the serological structures of all E. coli strains of at least one of the pair of samples have been determined; for example, if the antigenic structures of all five strains isolated from the lumen specimen have been determined, but not the structures of the five strains isolated from the faecal specimen, it has been concluded that the serotypes isolated from the lumen did not occur in the faeces.

The Escherichia coli strains of known antigenic structure encountered in the distal lumens of inflamed appendices were found also in the proximal lumens in 20 (87.0 ± 7.2 %) of 23

TABLE 14

THE OCCURRENCE OF THE SAME E. COLI SEROTYPE IN VARIOUS PARTS OF APPENDIX, IN PERITONEAL EXUDATE AND IN THE FAECES.

Type of appendicitis	Cases in which all serotypes of the lumen were present in the proximal part/no. of cases examined	Cases in which all serotypes of the wall were present in the lumen/no. of cases examined	Cases in which all serotypes in peritoneal exudate were present in the lumen/no. of cases examined	Cases in which all serotypes of the lumen were present in faeces/no. of cases examined	Cases in which all serotypes in peritoneal exudate were present in faeces/no. of cases examined
Ac. foc. A.	1/1	4/5	—	8/19	—
Phlegm. A.	3/3	26/30	—	19/55	—
Ulc.-phl. A.	3/4	13/18	—	8/27	—
Absc. A.	3/3	12/12	4/4	6/25	1/4
Gangr. A.	10/12	33/37	25/30	10/54	5/29
Pathol. material	20/23	88/102	29/34	51/180	6/33
Per cent	87.0 ± 7.2	86.3 ± 3.4	85.4 ± 6.1	28.3 ± 3.4	18.2 ± 6.8
Normal appendices	—	—	—	3/34 8.8 ± 4.9	—
Total	20/23	88/102	29/34	54/214	6/33
Per cent	87.0 ± 7.2	86.3 ± 3.4	85.4 ± 6.1	25.2 ± 3.1	18.2 ± 6.8

patients. Strains isolated from wall specimens were found in the lumen specimens of the same inflamed appendices in 88 (86.3 ± 3.4 %) of 102 patients. Strains isolated from peritoneal exudates were also isolated from the lumen specimens of 29 (85.4 ± 6.1 %) of 34 patients. When the uniformity of the E. coli strains in various parts of the same appendix was examined, it was noted that all serotypes isolated from the lumens occurred in equal frequency in the proximal part, all serotypes isolated from the walls occurred in equal frequency in the lumens, and all serotypes found in the peritoneal exudates occurred in equal frequency in the lumens. It is hence highly probable that an E. coli serotype spreads from the proximal part of the appendix to the lumen, from the latter into the wall, and through the latter into the peritoneal cavity.

When the serotypes found in the lumens and faeces of the appendicitis patients were compared, it was found that among the examined 180 patients there were only 51 (28.3 ± 3.4 %) in whom all serotypes found in the lumens of the inflamed appendices were also present in the faeces. For the patients with normal appendices, the percentage was even smaller, 8.8 ± 4.9. The E. coli serotypes isolated from peritoneal exudates were found also in the faeces of 18.2 ± 6.8 per cent of the patients.

When the E. coli serotypes in the lumen, wall tissue and faeces of each patient were compared, it was seen that almost always the same serotypes were isolated from the lumens and walls of the inflamed appendices, but seldom from both the lumens and the faeces of the patients, the difference between the frequencies being highly significant.

The patients in whom the uniformity of E. coli serotypes in the inflamed appendices and regional lymph nodes could be compared were only ten in number. In six of these the same serotype was found in both the lumen and the lymph node. Among the patients with normal appendices there were three in whom E. coli was found in the lymph nodes of the ileocoecal region and in these three patients the same serotype was isolated from the appendix lumen and the lymph node.

ESCHERICHIA COLI SEROTYPES EXHIBITING UNUSUAL BIOLOGICAL BEHAVIOUR

With only a relatively few exceptions the biochemical reactions of the Escherichia coli strains were characteristic of the species: they were mannitol, lactose, indole and methyl-red positive, produced gas, but not hydrogen sulphide, gave a negative Voges-Proskauer reaction and did not liquefy gelatin, utilize citrate or decompose urea. The Escherichia coli strains which deviated from the normal biochemical behaviour are listed together with their antigenic structures in Table 15.

All the examined Escherichia coli strains fermented mannitol. Five strains, all of type 1:51:7 * and from the same specimen, did not produce gas from mannitol and fermented lactose late, but

* The letters designating the type of antigen are omitted for brevity.

TABLE 15

ESCHERICHIA COLI STRAINS EXHIBITING ABNORMAL BIOCHEMICAL BEHAVIOUR.

Serotype	Number	Manitol/Gas	Lactose	Indole	H_2S	Gelatin	Urea	Simmonds	Voges-Proskauer	Methyl red	Adonitol	Dulcitol	Inositol	Salicin	Sucrose	Motility
16:1:—	2	+/+	+	+	—	—	—	+	—	+	—	+	—	$+^{4-7}$	—	--
1:1:—	1	+/+	+	—	—	—	—	—	—	+	—	+	—	—	+	—
6:5:—	3	+/+	—	+	—	—	—	—	—	+	—	+	—	—	+	—
8:50:—	4	+/+	+	—	—	—	—	—	—	+	—	$+^{3}$	—	$+^{3}$	+	—
1:51:7	3	+/+	—	+	—	—	—	—	—	+	—	+	—	—	—	+
9:33:—	10	+/+	+	—	—	—	—	—	—	+	—	—	—	—	—	—
2:5:—	4	+/+	—	+	—	—	—	—	—	+	—	+	—	—	+	—
18:5:—	3	+/+	—	+	—	—	—	—	—	+	—	$+^{7}$	—	$+^{3}$	+	—
23:21:H?	2	+/+	+	+	+	—	—	—	—	+	—	$+^{1-4}$	—	—	$+^{2}$	+
1:51:7	5	+/−	$+^{7-14}$	+	—	—	—	—	—	+	—	+	—	—	+	+
9:31:4	9	+/+	+	—	—	—	—	—	—	+	—	—	—	—	—	+
2:5:—	3	+/+	+	+	—	—	—	+	—	+	—	+	—	—	+	—
1:51:7	2	+/+	+	+	$+^{3-60}$	—	—	—	—	+	—	+	—	—	$+^{4}$	+

were motile, for which reason they were considered to belong to the E. coli group and not to the Alkalescens group. Thirteen strains in all were not found to ferment lactose; they were serotypes 2:5:—, 1:51:7, 6:5:— and 18:5—. All their other biochemical reactions were typical of E. coli. Strains that did not form indole were 24 in number and belonged to the serological types 1:1: —, 8:50: —, 9:31:4 and 9:33: —. Four strains produced hydrogen sulphide. None of the strains of the whole material liquefied gelatin, attacked urea, or gave a positive Voges-Proskauer or a negative methyl red reaction. Five strains that utilized citrate were of serotypes 2:5:— and 16:1:—. None of the strains fermented adonitol and inositol, and the ability to split dulcitol, salicin and sucrose was variable.

THE OCCURRENCE OF AGGLUTININS FOR ESCHERICHIA COLI IN THE SERA OF PATIENTS

To determine the participation of Escherichia coli in appendical processes, the occurrence of agglutinins for E. coli in the sera of appendicitis patients and in patients with healthy appendices was also investigated. The number of sera from appendicitis patients was 80 and the number from the other patients 20. One serum specimen was taken from each patient. In addition, the existence of antibodies for five of the most common O antigens found in this study and also for O 111 was examined in 44 control sera of persons who were not known to harbour any infection.

TABLE 16

THE OCCURRENCE OF E. COLI ANTIBODIES IN 44 CONTROL SERA AS ESTABLISHED BY BACTERIAL AGGLUTINATION (CENTRIFUGE METHOD) TESTS EMPLOYING SIX O ANTIGENS.

Agglutinin	Serum dilution					
	1/< 20	1/20	1/40	1/80	1/160	1/320
O1	19	—	9	11	4	1
O11	36	4	4	—	—	—
O15	39	1	3	1	—	—
O21	37	2	4	1	—	—
O8	40	1	1	2	—	—
O111	37	3	2	2	—	—

TABLE 17

THE OCCURRENCE OF E. COLI ANTIBODIES IN 44 CONTROL SERA AS EXAMINED BY HAEMAGGLUTINATION TESTS.

Agglutinin	Serum dilution				
	1/< 20	1/20	1/40	1/80	1/160
O1	17	3	14	9	1
O11	34	6	4	—	—
O15	29	7	7	1	—
O21	33	5	5	1	—
O8	20	2	14	7	1
O111	35	7	1	1	—

The results relating to agglutinins in the control sera are presented in Tables 16 and 17. From the first table it will be seen that the antibody titres obtained by bacterial agglutination using the centrifugation technique were 1:160 or 1:320 in only five sera and all these titres referred to the O 1 antigen. For all other antigens examined, the agglutinin titres were 1:80 or lower. The data in the latter table show that the highest titre found for E. coli antibodies by the haemagglutination test was 1:160. This

TABLE 18

THE OCCURRENCE OF E. COLI ANTIBODIES IN PATIENT SERA AS EXAMINED BY BACTERIAL AGGLUTINATION TESTS (CENTRIFUGE METHOD).

Agglutinin	No. of sera and patients	Serum dilution									
		1/< 20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Pathological material:											
Homologous O	80	28	5	8	9	9	4	5	9	1	2
„ K	80	76	3	—	—	1	—	—	—	—	—
„ H	42	35	4	1	1	1	—	—	—	—	—
O111 (control)	80	60	7	8	5	—	—	—	—	—	—
Normal appendices:											
Homologous O	20	8	2	2	6	2	—	—	—	—	—
„ K	20	20	—	—	—	—	—	—	—	—	—
„ H	16	14	1	1	—	—	—	—	—	—	—
O111 (control)	20	16	2	1	1	—	—	—	—	—	—

titre was observed in one case for each of the O antigens 1 and 8. In the majority of these sera no increase in agglutinin titre was established with either of the methods.

In Table 18 the occurrence of O, K and H agglutinins for the homologous E. coli lumen strains in the sera of the appendicitis patients and of patients with normal appendices is shown. It is seen that for 28 of the examined 80 appendicitis patients the O antibody titre was lower than 1:20, while for 21 patients the titre was 1:320 or higher, the highest titre being 1:5,120. In 20 of the

latter 21 patients the appendicitis was of a severe type, abscessed or gangrenous. In the remaining case the type was ulcero-phlegmonous and the antibody titre 1:5,120. Twelve of the 21 patients for whom the titre was 1:320 or higher were peritonitis cases. The E. coli lumen strains with respect to which the antibody investigations were performed were always found also in the peritoneal exudates.

O agglutinin titres of 1:320 were obtained for one patient with abscessed appendicitis (the antigenic structure of the strain isolated from the lumen was 15:14:4) and three patients with gangrenous appendicitis (2:2a,2b:1, 11:10:—, 1:1:—); in one of the latter patients perforation had occurred (11:10:—). O agglutinin titres of 1:640 were found for the following five appendicitis patients: one with perforative abscessed appendicitis (12:11:4) and four with gangrenous appendicitis (11:10:—, 1:1:— (2 cases), 1:51:—), two of them perforative (1:1:— and 1:51:—). In the last case also another strain (2:5:4) was isolated from the peritoneal exudate; the respective O agglutinin titre was 1:320. O agglutinin titres of 1:1,280 were obtained for nine patients: two with abscessed appendicitis (21:14:4, 15:14:—), in one of whom perforation had occurred (15:14:—); one with non-perforative gangrenous appendicitis (1:1:7) and six with perforative gangrenous appendicitis (6:5:—, 6:5:H?, 8:28:19, 8:42:19, 9:33:—, and 15:20:4). From the patient harbouring E. coli 15:20:4 a second serotype, 7:7:4, was isolated which gave an agglutinin titre of 1:320. A titre of 1:5,120 was obtained for one patient with abscessed appendicitis (15:14:—).

The highest K agglutinin titre recorded was 1:160 for a patient with perforative gangrenous appendicitis for whom the O agglutinin titre was 1:1,280; the serotype was 6:5:—. This was also the only patient for whom both the O and K agglutinin titres were high. The other K agglutinin titres were 1:20 in three patients and lower than 1:20 in 76 patients.

The highest recorded H antibody titre was 1:160. Titres lower than 1:20 were found for 35 of the examined 42 sera.

When the sera of the appendicitis patients were examined for agglutinins for the control strain O 111, titres higher than 1:80 were not obtained, and the titre 1:80 for only 5 sera.

Among the sera of the twenty patients with normal appendices there were only two with an O agglutinin titre of 1:160. In the sera of two patients the titre was below 1:20. All the K agglutinin titres were lower than 1:20. The highest H agglutinin titre was 1:40 and the highest O antibody titre for the control strain O 111 was 1:80.

TABLE 19

THE OCCURRENCE OF E. COLI ANTIBODIES IN PATIENT SERA AS EXAMINED BY E. COLI HAEMAGGLUTINATION TESTS.

Agglutinin	No. of sera and patients	Serum dilution							
		1/< 20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Pathological material:									
Homologous O	80	11	6	18	15	17	9	2	2
O111 (control)	80	62	3	14	1	—	—	—	—
Normal appendices:									
Homologous O	20	6	6	2	4	2	—	—	—
O111 (control)	20	14	3	—	3	—	—	—	—

All the sera referred to which were examined by bacterial agglutination tests were also investigated for the presence of E. coli antibodies by means of the haemagglutination test (Table 19). Haemagglutinin titres between 1:320 and 1:1,280 were established for 13 of the patients with diseased appendices. The antibody titres obtained by means of the bacterial haemagglutination test were on the average one or two tubes lower than the bacterial agglutination titres. The highest haemagglutinin titre recorded for patients with normal appendices was 1:160; this titre was obtained for two patients. The highest haemagglutinin titre recorded for the control strain O 111 in the two groups of patients was 1:80.

A comparison of the titres obtained for the patients with normal appendices and the control subjects shows that no differences

exist between the two groups. In both groups the highest bacterial agglutination and bacterial haemagglutination titres were 1:160 with the exception of one control subject for whom a titre of 1:320 for the O 1 antigen was recorded.

When the data for the appendicitis patients and the patients with normal appendices are compared, it is seen that high titres (1:1,280) were obtained in the bacterial agglutination tests for twelve patients of the former group, and in the bacterial haemagglutination tests for only two patients of this group, but in none of the tests in the latter group.

DISCUSSION

Escherichia coli is one of the most common bacteria in the large intestine and its appendix. The incidence (94.2 ± 1.5 %) of E. coli strains in acute appendicites found in the present study conforms with the results of previous investigations (65—100 %). No difference has been noted in the incidence of E. coli in inflamed and healthy appendices (Löhr and Rassfeld 1931, Eichhoff and Pfannenstiel 1931, Ewertsen and Knipschildt 1946). In the present study E. coli was found in equal frequency in the lumens, wall tissues and in parts of lumens proximal to lumen obstructions in inflamed appendices. In agreement with the findings of Bowers (1939), the incidence of bacteria in wall tissues of the appendix increased with the severity of the inflammation.

In the present study it was established, as Kauffmann (1954) Vahlne (1945) and Ewertsen and Knipschildt (1946) had previously, that the Escherichia florae in inflamed appendices possess special characteristics. The E. coli strains isolated from inflamed appendices are more often groupable into O groups 1—25 of the antigenic schema of Kauffmann—Knipschildt—Vahlne than those isolated from healthy appendices and appreciably more often than the strains isolated from faecal specimens. Vahlne has concluded that the E. coli strains more often belong to the same O group in pathological material (in 53 % of inflamed appendices) than in the normal material (in 46 % of normal appendices). It was established in the present study that 48.4 ± 3.2 per cent of appendicitis specimens contained only one serotype, whereas only 15.9 ± 2.8 per cent of faecal florae of appendicitis patients and 11.1 ± 4.7 per cent of the faecal florae of patients with healthy appendices contained only one serotype. In the remaining specimens several serotypes were present and in some specimens each of the five examined colonies was of a different serotype. The

percentage of E. coli florae of normal appendices containing a single serotype was 35.6 ± 7.1; this percentage does not deviate significantly from that for the inflamed appendices.

The most common O groups do not evidently differ in frequency in different countries since the most often encountered O groups 1, 2, 4, 6, 8, 9, 11, 15 and 21 have predominated also in the series of other investigators (Kauffmann 1954, Vahlne 1945, Ewertsen and Knipschildt 1946, Mondolfo and Hounie 1947, and Grönroos 1957).

On the other hand, no E. coli serotype was encountered more often than any other in inflamed appendices, and hence it cannot be considered that there is a definite serotype specific for appendicitis. Vahlne (1945), however, on the basis of a study of the incidence of strains of O group 9, came to the conclusion that certain serologically defined E. coli types must primarily be taken into account in appendicitis. Although a number of serotypes were isolated from the appendices of this study that were not found in healthy appendices or faecal specimens, the differences in frequency are not large enough to support Vahlne's opinion.

No differences were noted in the serological properties of the E. coli strains isolated from the various forms of appendicitis examined: acute focal, phlegmonous, ulcero-phlegmonous, abscessed and gangrenous. This is quite understandable as it is generally held (Aschoff 1930 and Büchner 1955) that all these different forms represent different stages of the same disease. Each removed inflamed appendix reveals histopathological features that reflect the stage of the disease at the time the operation was performed.

An interesting observation is that the same Escherichia coli serotype was isolated from different parts of the same inflamed appendix, the part of the appendix proximal to a lumen obstruction, the lumen of the distal inflamed part and the appendix wall tissue, and, in peritonitis, from the peritoneal exudate, in nearly 90 per cent of the cases. On the other hand, the serotype isolated from the lumen was relatively less frequently found in the faeces of the patient in question (28.3 ± 3.4 %). In the patients with healthy appendices, the serotype from the lumen was only rarely found in the faeces (8.8 ± 4.9 %). This finding suggests that the Escherichia flora in the appendix region of

TABLE 20

RELATIVE INCIDENCE (IN PERCENT) OF E. COLI STRAINS OF O GROUPS 1—25 IN DIFFERENT SERIES.

Sources	Authors													
	Kauffmann 1944	Vahlne 1945	Ewertsen & Knipschildt 1946	Mondolfo & Hounie 1947, 1948	Wramby 1948	Scire 1950	Parvis & Grosso 1953	Levanto 1954	Grönroos, Mustakallio & Virtanen 1955	Grönroos 1957	ørskov 1956	Fey 1955	Kuhnyine, Schwanner & Hamar 1955	Present series
Human														
Pathological														
Peritonitis	62	73	75				53							88
Appendicitis		78	74			46	65						44	77
Urinary infections		61					26		31				52	
Cholecystitis		44					23		33					
Suppurative conditions														
Achlorhydric gastric juice								33						
Infantile diarrhoea patients										29	38			
Faeces (in appendicitis)		55												49
Different sources				64										
Normal														
Faeces	14	42	51	64		61	24						33	
Faeces of patients with normal appendices		52												42
Faeces of infants										33	40			
Normal appendices		68	65				36							57
Water				32			6							
Animal														
Various infections					40		11					40		
Normal faeces							17					5		
No. of strains examined	92	5542	2868	1279	5961	225	1048	82	483	2645	764	1919	300	3500

the digestive tract differs from the flora of the aboral part of the large intestine. On the other hand, as far as the oral part of the digestive tract is concerned, it is known that E. coli is only rarely, if at all, found in acid gastric juices (Henning 1930, Korttila 1951, Levanto 1954). Thirty-three per cent of the E. coli strains Levanto (1954) isolated from achlorhydric gastric juices belonged to O groups 1—25. The percentage of O-groupable strains among E. coli strains isolated from inflamed appendices in the present study, 77 per cent, is much higher and also suggests that the E. coli florae in the oral part of the digestive tract and in the appendical region differ from each other. E. coli strains of O groups 1—25 have been isolated in only low frequency from other sources than inflamed appendices, e.g. from infantile diarrhoea (Ørskov 1956, Grönroos 1957) and from various animal infections (Wramby 1948, Frey 1955; see Table 20).

In order to determine the aetiological significance of E. coli in appendicitis it would have been important to determine whether any increase occurs in the agglutinin titres with respect to the homologous appendical strains during the development of appendicitis, but this was not possible in the present study. A study was, however, made of the agglutinin titres in single serum specimens. It was found that the O agglutinin titres were clearly higher in 10 per cent of the patients with uncomplicated appendicitis than in the control subjects. The highest titres recorded were 1:5,120.

The results of the present study show that the E. coli florae in inflamed and healthy appendices are much more uniform than faecal florae. The same serotype was isolated from different parts of the inflamed appendix and from the peritoneal exudate in nearly 90 per cent of the appendicitic patients. Agglutinins for the homologous luminal strains were found in the sera of some patients. By taking these observations into account it may prove possible to shed more light upon the part played by micro-organisms in the genesis of appendicitis.

CONCLUSIONS

1. When the Escherichia coli florae in inflamed and normal appendices were compared, it was found that the proportion of O-groupable strains is clearly larger in the former. The florae of inflamed appendices were serologically highly uniform since a single E. coli serotype predominated in nearly half of the cases. The difference in this respect between inflamed and normal appendices is not so clear that it allows definite conclusions to be drawn. The difference is, however, pronounced when florae of inflamed appendices are compared with faecal florae. The faecal strains definitely less frequently belonged to the first twen-tyfive O groups of the antigenic schema, were rarely O-inagglutinable and were less often of the same serotype than the strains in the inflamed appendices. It is noteworthy that the serotype or serotypes encountered in different parts of the inflamed appendices were seldom isolated from the faeces specimens of the patients. Likewise, the serotypes found in healthy appendices were only rarely encountered in faeces specimens from the respective subjects.

2. The Escherichia florae in various regions of inflamed appendices, i.e. in the parts proximal to lumen obstructions, in the lumens, in the wall tissues, and in peritoneal exudates, are serologically uniform since the strains in all groups of specimens were found to belong to O groups 1—25 of the antigenic schema in approximately the same percentage, and approximately the same proportion of the strains was O-inagglutinable. In addition, all E. coli strains isolated from the various specimens were of one serotype in nearly half of the cases. The most important observation, however, is that in about 90 per cent of patients the same E. coli serotypes were found in the lumens proximal and distal to obstructions and in wall tissues of the inflamed appendices and in the peritoneal exudates.

3. Histopathologically different forms of appendicitis were not found to differ from each other in respect of the serological properties of the Escherichia strains isolated from them.

4. It was only infrequently that the O agglutinin titres for the homologous Escherichia coli strains isolated from the appendical lumens were higher in the sera of appendicitis patients than in the sera of patients with normal appendices or in the control sera. Furthermore, the appendicitis patients in question suffered from the most severe forms of appendicitis. Of the 21 appendicitis patients with high O agglutinin titres, 12 had peritonitis. The highest O agglutinin titre in uncomplicated appendicitis and in peritonitis was 1:5,120. Antibody titres obtained by bacterial haemagglutination tests were lower than those obtained by bacterial agglutination tests in which centrifugal separation was employed. The K agglutinin titre was in only one appendicitis patient higher than in patients with normal appendices and in the control subjects. All the H agglutinin titres were low.

SUMMARY

The aim of the present study was to determine whether and in what degree the Escherichia coli florae in acutely inflamed and healthy appendices and in faecal specimens of the patients differ from each other. In addition, the serological properties of the Escherichia strains in various parts of the appendices and in the peritoneal exudates were investigated. The presence in the sera of the patients of antibodies homologous for Escherichia strains isolated from the appendical lumens was also studied.

The total number of excised appendices examined was 312. Specimens were taken from these as follows: 310 lumen specimens, 35 specimens from parts proximal to lumen obstructions, 289 wall tissue specimens and 72 peritoneal exudates. From these specimens, 3,500 Escherichia coli strains were isolated. Sera of 80 patients were examined for the presence of agglutinins homologous for the E. coli strain isolated from the appendical lumen in each case. The results were the following:

1. The appendices were grouped according to their *histopathological appearance* into 24 acute focal, 82 plegmonous, 45 ulcero-phlegmonous, 37 abscessed (5 of these perforated), 69 gangrenous (35 of these perforated) and 53 normal appendices. Two cases of tuberculous appendices were encountered, but the results for these are not included below.

2. Bacterial growth. None of the lumen specimens or of the specimens from parts proximal to lumen obstructions in the inflamed appendices and only one of the lumen specimens from healthy appendices was free of bacteria. No bacteria grew from 39.4 ± 3.3 per cent of the wall tissues from the inflamed appendices; the corresponding percentage for the mild forms of appendicitis (acute focal, phlegmonous and ulcero-phlegmonous) was 48.6 ± 4.2 per cent, and for the severe forms (abscessed and gangrenous) 26.3 ± 4.4 per cent. All the wall specimens from

normal appendices were free of bacteria. The proportion of peritoneal exudates that grew no bacteria was 40.3 ± 6.0 per cent and that of the lymph nodes, 81.5 ± 3.6 per cent. All faecal specimens yielded a luxuriant bacterial growth.

3. The Incidence of Escherichia coli. In the group of appendicitis patients Escherichia coli strains were isolated

from 94.2 ± 1.5 per cent of the lumen specimens,
from 94.2 ± 4.1 per cent of the proximal lumen specimens,
from 88.4 ± 2.7 per cent of the wall tissue specimens,
from 97.5 ± 2.5 per cent of the peritoneal exudates, and
from 94.8 ± 1.5 per cent of the faecal specimens.

E. coli strains grew from 86.5 ± 4.9 per cent of the lumen specimens from normal appendices and from 97.7 ± 2.2 per cent of the faecal specimens from patients with normal appendices. The frequencies for the various groups of specimens do not differ significantly.

4. The O groupability of the Escherichia coli strains. Neither the strains isolated from the various forms of inflamed appendices nor the strains isolated from lumens ($76.7 \pm 1.3\%$ O-groupable), proximal parts ($80.0 \pm 3.3\%$) and wall tissue specimens ($77.1 \pm 1.7\%$) from the inflamed appendices differed in their O groupability. 88.0 ± 2.5 per cent of the peritonitis strains, 68.7 ± 6.5 per cent of the strains from lymph nodes and 49.5 ± 1.7 per cent of the strains from the faecal specimens from the appendicitis patients could be entered into O groups 1—25. The percentages of O-groupable strains isolated from the appendical lumens and the faecal specimens of patients with normal appendices were 57.0 ± 3.2 per cent and 41.8 ± 3.5 per cent, respectively. From these figures it is seen that the inflamed appendices and peritoneal exudates contained larger proportions of strains belonging to O groups 1—25 than normal appendices, and much larger proportions than were found in faecal specimens from both groups of subjects. Of all the 3,500 E. coli strains isolated, 66.9 ± 0.8 per cent could be identified as to their O antigen.

5. No O group or serotype was found to predominate over any other. The most common O groups were 1, 15, 2, 9, 8, 21, 4, 11 and 6. Strains conforming with the antigenic schema amounted to 56.7 ± 1.2 per cent of all the serologically identified strains.

6. O-inagglutinable Escherichia coli strains amounted to 93.7 ± 0.8 per cent of the strains isolated from inflamed appendices, to 88.9 ± 2.7 per cent of the strains from normal appendices, to 84.8 ± 1.7 per cent of the strains from faeces specimens from appendicitis patients, and to 84.7 ± 3.9 per cent of the strains from patients with normal appendices.

7. The most frequently encountered K antigens were 1 L, 5 L, 20 L, 14 L, 51 L and 10 L. The first two antigens were found in 41.8 ± 3.4 per cent of the strains of the lumen specimens from appendicitis patients. However, the corresponding frequencies for faeces and normal appendix lumen specimens were of the same order.

8. Haemolytic strains comprised 15.0 ± 0.7 per cent of the strains from appendicitic lumen specimens, 12.0 ± 1.5 per cent of the strains from faeces specimens of appendicitis patients, 12.2 ± 2.8 per cent of the strains from normal appendices and 13.0 ± 3.7 per cent of the strains from faecal specimens of patients with normal appendices. The differences between the percentages are not significant.

9. The percentages of specimens from appendicitis patients that contained strains of only one serotype were: lumens, 48.4±3.2 %; wall tissues, 57.4 ± 4.4 per cent; peritoneal exudates, 61.6 ± 7.8 per cent; faecal specimens, 15.9 ± 2.8 per cent. The first three percentages do not differ significantly from each other, but the faecal specimens are seen to be much less uniform serologically than the other specimens. Strains of one serotype were found in 35.6 ± 7.1 per cent of the lumen specimens and in 11.1 ± 4.7 per cent of the faecal specimens from patients with normal appendices; the two groups of specimens thus differ in the degree of uniformity of their strains. There is, however, no significant difference in the serological uniformity of the flora in inflamed and normal appendices.

10. The same E. coli serotype was isolated from both the distal and proximal lumens of 87.0 ± 7.2 per cent of the obstructed inflamed appendices. The same serotype was found in the lumen and wall tissues in 86.3 ± 3.4 per cent and in the peritoneal exudate and the lumen in 85.4 ± 6.1 per cent of the appendicitis patients. It thus appears that the same serotypes are found in equal frequency in all parts of the inflamed appendix and the peritoneal

exudate. On the other hand, the same serotype was isolated from the appendix lumen and the faeces in only 28.3 ± 3.4 per cent of the appendicitis patients and in only 8.8 ± 4.9 per cent of the patients with normal appendices. The serotype isolated from the peritoneal exudate was found in the faeces in only 18.2 ± 6.8 per cent of the peritonitis patients.

11. O antibody titres for homologous Escherichia coli strains that were higher than the titres in the sera of patients with uninflamed appendices and of healthy controls were found for 21 of the sera from 80 appendicitis patients.. All the 21 high antibody titres were recorded for sera from patients with severe forms of appendicitis, of whom 12 had peritonitis. For 12 sera the titre was between 1:1,280 and 1:5,120; the latter titre was recorded for two patients of whom one had peritonitis. A K agglutinin titre (1:160) higher than the titres found for sera of the two control groups was observed in only case. High A antibody titres were not encountered. Antibody titres determined by the bacterial haemagglutination test were all lower than the titres obtained in bacterial agglutination tests in which centrifugation was employed.

REFERENCES

ALAMERI, R., and PÖYHÖNEN, P.: Johdatus tilastolliseen tutkimukseen. 2. painos. Helsingin Yliopisto. Helsinki, 1954. Pp. 58, 60, 61, 89.

ALTEMEIER, W. A.: Ann. Surg. 1938:*107*:517.

ASCHOFF, L.: Die Wurmfortsatzentzündung. Gustav Fischer. Jena, 1908.

ASCHOFF, L.: Der Appendicitische Anfall. Julius Springer. Berlin und Wien, 1930.

BAGGER, S. W., and MIKKELSEN, O.: Hospitalstid. 1924:*67*:289.

BALICE, G.: Riforma med. 1937:*53*:883.

BITTER, L., and GUNDEL, M.: Ztschr. Hyg. 1925:*104*:203 and 752.

BOWER, J. O., BURNS, . C., and MENGLE, H. A.: Surgery 1938:*3*:645.

BOWERS, W. F.: Arch. Surg. 1939:*39*:362.

BRAUN, O. H., SEELIGER, H., and WAGNER, N.: Ztschr. Kinderh. 1954: *75*:50.

BURK, A.: Zentralbl. Bakt. Orig. 1908:*45*:577.

BÜCHNER, F.: Spezielle Pathologie. Urban - Schwarzenberg. München - Berlin, 1955. Pp. 238-246.

CRAIGIE, J.: J. Immunol. 1931:*21*:417.

DIAZ MARTIN, L.: Med. colon. 1946:*7*:484.

DUDGEON, L. S., and MITCHINER, P. H.: Brit. J. Surg. 1923:*11*:676.

DURHAM, H. E.: cited by Knipschildt.

EICHHOFF, E., and PFANNENSTIEL, W.: Beitr.klin. Chir. 1931:*151*:172.

EWERTSEN, H. W.: Dyreexperimentelle Undersøgelser over Colibacillernes Patogenitet og Effekten af Coliserum. Arnold Busck. Copenhagen, 1946. Diss.

EWERTSEN, H. W., and KNIPSCHILDT, H. E.: Acta path. et microbiol. scandinav. 1946:*23*:170.

EWING, W. H., TATUM, H. W., DAVIS, B. R., and REAVIS, R. W.: Studies on the Serology of the Escherichia Coli Group. Communicable Disease Center. Atlanta, Georgia, 1956.

FARRIS, J., and ROMACK, H.: Surgery 1947:*22*:305.

FEY, H.: Serologische, biochemische und biologische Untersuchungen an Stämmen aus boviner Colimastitis mit spezieller Berüchsichtigung der Coli-Säuglingsenteritis. Erg. Hyg. Bakt. 1955:*29*: Suppl. 4. Diss.

GAETHGENS, W.: München. med. Wchnschr. 1906:1351.

GARA, P. F. DE: Science 1939:*90*:378.

GRABER, J., and BONNEFOI, A.: Ann. Inst. Pasteur 1946:*72*:745.

GRÖNROOS, J. A.: Investigations on Certain Escherichia Coli Sero-Types with Special Reference to Infantile Diarrhoea. Ann. med. exper. et biol. Fenniae 1954:*32*:Suppl. 4. Diss.

GRÖNROOS, J. A.: Ann. med. exper. et biol. Fenniae 1957:*35*:Suppl. 2.

GRÖNROOS, J. A., MUSTAKALLIO, E., and VIRTANEN, S.: Ann.med.exper. et biol. Fenniae 1955:*33*:133.

HAYASHI, K.: Ztschr. Immunitätsforsch. 1938:*92*:118.

HEES, H.: Zentralbl. Bakt. Orig. 1926:*99*:19.

HEILE, B.: Mitt. Grenzg. Med. Chir. 1913:*26*:345.

HENNING, N.: Arch. Verdauungskr. 1930:*47*:1.

HIROSE, H.: J. Exper. Med. 1930:*15*:524.

HUBER, K.: Wien. med. Wchnschr. 1950:*100*:715.

HUSTED, E.: Nord. med. 1942:*13*:96.

KAUFFMANN, F.: Die Bakteriologie der Salmonella-Gruppe. Ejnar Munksgaard. Copenhagen, 1941.

KAUFFMANN, F.: Acta path. et microbiol. scandinav. 1943:*20*:21.

KAUFFMANN, F.: Acta path. et microbiol. scandinav. 1944:*21*:20, 46 and 72.

KAUFFMANN, F.: Schweiz. Ztschr. allg. Path. 1948:*11*:553.

KAUFFMANN, F.: Enterobacteriaceae. Ejnar Munksgaard. Copenhagen, 1954.

KAUFFMANN, F., and PERCH, B.: Acta path. et microbiol. scandinav. 1948:*25*:507.

KAUFFMANN, F., and VAHLNE, G.: Acta path. et microbiol. scandinav. 1945:*22*:119.

KAUFFMANN, F., ØRSKOV, F., and EWING, W. H.: Int. Bull. Bact. Nomenclat. Taxonomy 1956:*6*:63.

KELLY, cited by Altemeier.

KEMAL, M.: Deutsche med. Wchnschr. 1934:*60*:504.

KNIPSCHILDT, H. E.: Undersøgelser over Coligruppens Serologi med saerlig Henblik paa Kapselformene. Arnold Busck. Copenhagen, 1945. Diss.

KORTTILA, K.: Bacteriological Studies in Cases of Perforated Gastroduodenal Ulcer with Reference to Clinical and Pathological Aspects. Acta chir. scandinav. 1951:Suppl. 163. Diss.

KRÖGER, E., and GILLESEN, J.: Zentralbl. Bakt. 1950:*155*:115.

KROGIUS, A.: Forhandlingerne ved Nordisk kirurgisk forening. 1900:109.

KROGIUS, A.: Über die vom Processus vermiformis ausgehende diffuse eitrige Peritonitis und ihre chirurgische Behandlung. Gustav Fischer. Jena, 1901.

KUBINYIÉ-SCHWANNER, M., and HAMAR, M.: Orv. hetil. 1955:*96*:628.

LANZ, O., and TAVEL, T.: Rev. chir. 1904:*30*:43 and 215.

LEVANTO, A.: A Study of Gastric Escherichia Strains with Special Reference to Pernicious Anaemia. Ann. med. int. Fenniae 1954:*43*:Suppl.20. Diss.

LÖHR, W., and RASSFELD, I.: Die Bakteriologie der Wurmfortsatzentzündung. Georg Thieme. Leipzig, 1931.

MCCLURE, R., and ALTEMEIER, W.: Ann. Surg. 1937:*105*:800.

MCWILLIAMS, C. A.: Ann. Surg. 1910:*61*:909.

MELENEY, F. L., HARVEY, N. D., and ZAYTSEFF-JERN, H.: Arch. Surg. 1931:*22*:1.

MELENEY, F. L., OLPP, J., HARVEY, H. D., and ZAYTSEFF-JERN, H.: Arch. Surg. 1932:*25*:709.

MIKKELSEN, O.: Undersøgelser over de hos Mennesket forekommende Former af Bacterium Coli. Kandrup & Wunsch. Copenhagen, 1927. Diss.

MONDOLFO, H., and HOUNIE, E.: Prensa med. Argent. 1947:*34*:328.

MONDOLFO, H., and HOUNIE, E.: Igiene Sanita Pubblica, Salerno. 1948:*4*:3.

NETER, E., and MILCH, E.: J. Infect. Dis. 1940:*66*:166.

NETER, E., BERTRAM, L. F., ZAK, D. A., MURDOCK, M. R., and ARBESMAN, C. E.: J. Exper. Med. 1952:*96*:1.

NETER, E., BERTRAM, L. F., and ARBESMAN, C. E.: Proc. Soc. Exper. Biol. & Med. 1952:*79*:255.

NETER, E., ZALEWSKI, N. J., and FERGUSON, W. W.: Proc. Soc. Exper. Biol. & Med. 1953:*82*:215.

NETER, E., GORZYNSKI, E. A., ZALEWSKI, N. J., RACHMAN, R., and GINO, R. M.: Am. J. Pub. Health 1954:*44*:49.

NIGAM, R.: Indian M. Gaz. 1947:*82*:709.

ØRSKOV, F.: Acta path. et microbiol. scandinav. 1956:*39*:137.

ØRSKOV, F.: Escherichia Coli. Om Typebestemmelse, specielt med Henblick på Stammer fra infantil Diarrhoea. Arnold Busck. Copenhagen, 1956. Diss.

PARVIS, D. and GROSSO, E.: Boll. Ist. Sieroter. Milan. 1953:*32*:194.

RUNEBERG, B.: Arbeiten a.d. Pathologischen Institut der Universität Helsingfors. Band II, 1908.

SCHENKEN, J. R., ANDERSON, T. R., and COLEMAN, F. C.: Am. J. Clin. Pathology 1956:*26*:352.

SCHÖNBAUER, L.: Arch. klin. Chir. 1951:102.

SCIRE, F.: Sicilia med. 1950:*7*:272.

SJÖSTEDT, S.: Pathogenicity of Certain Serological Types of B. Coli. Acta path. et microbiol. scandinav. 1946:Suppl.LXIII. Diss.

SMITH, Th.: J. Exper. Med. 1927:*46*:141.

SOAVE, F.: Pathologia Sperimentale 1950:*37*:143.

VAHLNE, G.: Serological Typing of the Colon Bacteria with Special Reference to the Occurrence of B. Coli in Man under Normal and Pathological Conditions, Particularly in Appendicitis. Acta path. et microbiol. scandinav. 1945:Suppl.LXII. Diss.

VAHLNE, G.: Nord. med. 1945:*27*:1628.

VICK, M.: cited by Bower et alii.

WANGENSTEEN, O. H., and BOWERS, W. F.: Arch. Surg. 1934:*34*:496.

WANGENSTEEN, O. H., BUIRGE, R. E., DENNIS, C., and RITCHIE, W.: Ann. Surg. 1937:*106*:910.

WANGENSTEEN, O. H., and DENNIS, C.: Ann. Surg. 1939:*110*:629.

WANGENSTEEN, O. H., and DENNIS, C.: Surg. Gynec. & Obst. 1940: *70*:799.

WARREN, S.: Am. J. Path. 1925:*1*:241.

WEINBERG, M., PIEROT, A., DAVESNE, J., and RENARD, C.: Ann. Inst. Pasteur 1928:*42*:1167.

WIDHOLM, O.: Studies on Escherichia Coli Hemolysins and Antihemolysins. Ann.med. exper. et biol. Fenniae 1953:*31*:Suppl. 5. Diss.

WRAMBY, G.: Investigations into the Antigenic Structure of Bact. Coli Isolated from Calves. Acta path. et microbiol. scandinav. 1948: Suppl.LXXVI. Diss.